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SELECTIVE INHIBITORS AND ALLOSTERIC ACTIVATORS OF SPHINGOSINE KINASE

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CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to Provisional Application No. 61759393, filed January 31, 2013, the disclosure of which is incorporated by reference in its entirety.

BACKGROUND OF THE INVENTION

Sphingosine kinase (SK) catalyzes the transfer of a phosphate group of ATP to sphingosine (Sph), forming sphingosine 1-phosphate (S1P). S1P is a bioactive lipid that mediates inflammation and regulates cell proliferation and cell motility. SK plays an important role in the balance between S1P, which is anti-apoptotic, and the pro-apoptotic sphingolipid precursors sphingosine and ceramide. Sphingosine kinase exists as two isoforms: sphingosine kinase 1 (SK1) and sphingosine kinase 2 (SK2). The isoforms are encoded by distinct genes and differ in their biochemical properties, subcellular localization, and function.

SK is elevated in many human diseases, including cancers, pulmonary fibrosis, inflammatory diseases such as asthma and atherosclerosis, and infectious diseases. Therefore, reduction of the levels of S1P can prevent hyperproliferation of cells that lead to pulmonary arterial hypertension and cancer. On the other hand, there is evidence that raising the level of intracellular S1P by activation of SK1 in response to Transforming Growth Factor-β has a potential anti-fibrotic effect in kidney (see: Ren, S., Babelova, A., Moreth, K., Xin, C., Eberhardt, W., Doller, A., Pavenstädt, H., Schaefer, L., Pfeilschifter, J., Huwiler, A. (2009) Transforming growth factor-beta2 upregulates sphingosine kinase-1 activity, which in turn attenuates the fibrotic response to TGF-beta2 by impeding CTGF
expression. *Kidney International* 76, 857-867). SK1 and Connective Tissue Growth Factor (CTGF) are up-regulated in podocytes from streptozotocin-induced diabetic mice and the disease is exacerbated in SK1-deficient mice, as evidenced by enhanced albuminuria and CTGF expression compared to wild type mice (Ren et al. 2009).


As SK1 and SK2 are potential and promising targets for cancer chemoprevention, a number of SK inhibitors have been prepared in order to reduce cancer cell survival but in general only very few have been found to be isoform selective or are metabolically stable. Therefore, there is an unmet need for selective inhibitors of either SK1 or SK2, but not both.

**SUMMARY OF THE INVENTION**

The present invention provides compounds, methods for their preparation, compositions containing the compounds, and methods of use of the compounds to selectively inhibit either of the two SK isoforms, to induce proteasomal degradation of SK1, to inhibit DNA synthesis in mammalian pulmonary smooth muscle cells and cancer cells, to induce apoptosis in these cells, and to activate SK1 for indication as an anti-fibrotic agent.

The present invention also provides methods of use of the compounds for the treatment of disorders and diseases associated with the activities of sphingosine kinase isoforms 1 and 2.
The present invention also provides for therapeutic agents for cancer, vascular remodeling in pulmonary hypertension, and fibrotic disease through the modulation of the activity of sphingosine kinases.

Also provided in this invention are compounds that activate SK1 and methods for treatment of disorders such as fibrosis, where intracellular S1P is anti-fibrotic (see: Pyne, S., Dubois, G., and Pyne, N.J. (2013) Role of sphingosine 1-phosphate and lysophosphatidic acid in fibrosis. Biochimica Biophysica Acta 1831, 228-238).

In one aspect, the present invention provides for a compound of formula I:

(I)

in which $R^1$ is a hydrogen, lower alkyl, or lower alkoxy; $R^2$ and $R^3$ are independently hydrogen, $C_1$-$C_{10}$ alkyl, or $-C(X)NHAr$; $X$ is oxygen or sulfur; Ar is aryl or heteroaryl group; $R^4$ is a hydrogen, or hydroxyl; $R^5$ is a $C_mH_{2m+1}$ straight-chain or branched alkyl, $C_2$-$C_{20}$-alkenyl, $C_2$-$C_{20}$-alkynyl, or $C_1$-$C_{20}$-alkoxy; $m$ is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20; bond A is a single or double bond; with the proviso that when $R^2$ and $R^3$ are hydrogen, $R^1$ is not methyl; and with the proviso that when A is a trans-double bond, and when the configuration at C-2 is $S$ and when the configuration at C-3 is $R$, $R^1$ is not hydroxymethyl (CH$_2$OH).
In another aspect, the present invention provides for a compound of formula II:

\[(II)\]

in which \(R^1\) is \(C_3-C_{12}\) alkyl, aryl, cycloalkyl, heterocyclyl, or heteroaryl, having a single cyclic ring or multiple condensed rings, quaternary ammonium group, or

\[
\begin{align*}
\text{Z} & \quad \text{OH} \\
\text{OH} & \quad \text{OH} \\
\text{N} & \quad \text{3} \\
\text{3} & \quad \text{Z} \\
\end{align*}
\]

in which \(Z\) is –OH, F, Br, Cl, or I. \(R^2\) is straight-chain or branched alkyl \(C_nH_{2m+1}\), \(C_2-\)
\(C_{20}\)‐alkenyl, \(C_2-C_{20}\)‐alkynyl, \(C_{1}-C_{20}\)‐alkoxy, or \(C_2-C_{20}\)‐alkyl-substituted heterocycle; \(m\) is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20; \(n\) is 0, 1, 2, 3, 4, or 5. \(W\) is –\(\text{CH}_2\) or oxygen; and \(X\) and \(Y\) are independently hydrogen, \(C_1-C_4\)-alkyl, or \(X\) and \(Y\) taken together are oxygen or sulfur.
In yet another aspect, the present invention provides for a compound of formula III:

(III)

![Chemical structure of compound III]

in which R\(^1\) is a C\(_{m}\)H\(_{2m+1}\) straight-chain or branched alkyl, C\(_2\)-C\(_{20}\)-alkenyl, C\(_2\)-C\(_{20}\)-alkynyl, or C\(_1\)-C\(_{20}\)-alkoxy; m is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20; R\(^2\) is hydrogen, hydroxyl, or C\(_1\)-C\(_{20}\)-alkoxyl; R\(^3\) is oxygen or sulfur; R\(^4\) is aryl or heteroaryl; and X and Y are independently NH or oxygen.

In another aspect, the present invention provides a method for selectively inhibiting SK1 in a cell by administering the compounds described above.

In another aspect, the present invention provides a method for selectively inhibiting SK2 in a cell by administering the compounds described above.

In another aspect, the present invention provides a method for selectively activating SK1 in a cell by administering the compounds described above.

In another aspect, the present invention provides a method of inducing apoptosis in a cell by administering the compounds described above.
In yet another aspect, the present invention provides a method of selectively inhibiting SK1 in a cell by administering a compound of formula IV:

(IV)

In

, or cis-sphingosine to said cell.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 depicts the effect of compounds RB-001 – RB-022 on SK1 or SK2 activity. SK1 activity was measured using 3 µM sphingosine and 250 µM ATP. SK2 activity was assayed using 10 µM sphingosine and 250 µM ATP (n = 3 for each compound, results expressed as % of the control ± S.D). RB series compounds were used at 50 µM.

FIG. 2 depicts the effect of compounds on SK1 or SK2 activity. SK1 activity was measured using 3 µM sphingosine and 250 µM ATP. SK2 activity was assayed using 10 µM sphingosine and 250 µM ATP (n = 3 for each compound, results expressed as % of the control ± S.D).
FIG. 3 depicts the effect of compounds RB-023-RB-065 on SK1 or SK2 activity. SK1 activity was measured using 3 µM sphingosine and 250 µM ATP. SK2 activity was assayed using 10 µM sphingosine and 250 µM ATP (n = 3 for each compound, results expressed as % of the control ± S.D).

FIG. 4 depicts the evaluation of compounds as putative substrates of SK1 and SK2. SK1 and SK2 activity was measured using 50 µM compound and 250 µM ATP in the absence of Sph (n = 3 for each compound). The results are expressed as % of control ± S.D. Control = activity using Sph alone (3 µM for SK1 and 10 µM for SK2) and is represented as 100%, against which each compound alone is compared.

FIG. 5 depicts the effect of inhibitors on SK1 or SK2 activity. SK1 activity was measured using 3 µM sphingosine and 250 µM ATP. SK2 activity was assayed using 10 µM sphingosine and 250 µM ATP (n = 3 for each compound, results expressed as % of the control ± S.D).

FIG. 6 depicts the evaluation of compounds as putative substrates of SK1 and SK2. SK1 and SK2 activity was measured using 50 µM compound and 250 µM ATP in the absence of Sph (n = 3 for each compound); results are expressed as % control ± S.D. Control = activity using Sph alone (3 µM for SK1 and 10 µM for SK2) and is represented as 100% against which each compound alone is compared.

FIG. 7 depicts the assessment of the effects of PF-543 (10 nM and 100 nM, 24 h), VPC96091 (300 nM, 24 h), and 55-21 (100 nM and 1 µM, 24 h) on [³H]-thymidine incorporation into DNA in PASMC. Results are expressed as % control ± S.D. of control (n = 3); the control is set to 100%. *** p <0.05 versus control.
FIG. 8 depicts the effects of azido alcohol and azido fluoro analogues of (S)-FTY720 vinylphosphonate on SK1 activity. The substrates were 3 µM sphingosine (which corresponds to the K_m of SK1) and 250 µM ATP (n = 3, results expressed as % control ± S.E.). The control is set at 100% and represents the SK1 activity against sphingosine alone.

FIG. 9 depicts the effects of 55-21 (A), F-02 (B) and RB-005 (C) on SK1 expression. Pulmonary arterial smooth muscle cells (PASMC) were treated with or without MG132 (10 µM, 30 min) before 55-21, F-02, or RB-005 (all at 10 µM, 24 h). Cell lysates were western blotted with anti-SK1 and -actin antibodies. Results are representative of three experiments.

**DETAILED DESCRIPTION**

The present invention provides compounds, methods for their preparation, compositions containing the compounds, and methods of use of the compounds to selectively inhibit either of the two SK isoforms, to induce proteasomal of SK1, to inhibit DNA synthesis in mammalian pulmonary smooth muscle cells and cancer cells, to induce apoptosis in these cells, and to activate SK1 for indication as an anti-fibrotic agent.
In one aspect, the present invention provides for the compound of formula I:

\[ R^1 \text{ represents a hydrogen, lower alkyl, or lower alkoxy; } R^2 \text{ and } R^3 \text{ independently represent hydrogen, C}_1^{10}\text{ alkyl, or } \text{–C(X)NHAr, in which X is oxygen or sulfur; } \]

The term “alkyl” refers to a saturated, linear or branched hydrocarbon moiety, such as –CH$_3$ or –CH(CH$_3$)$_2$. The term “lower alkyl” refers to straight or branched chain moiety having up to eight carbon atoms. The term “alkoxy” refers to the group “alkyl-O-” which includes, by way of example, methoxy, ethoxy, $n$-propoxy, iso-propoxy, $n$-butoxy, $t$-butoxy, $sec$-butoxy, $n$-pentoxy, and the like. The term “Ar” refers to aryl or heteroaryl group. Aryl refers to an aromatic carbocyclic group having at least one aromatic ring or multiple condensed rings in which at least one ring is aromatic. Heteroaryl refers to an aromatic ring system containing at least one ring heteroatom selected from, for example, oxygen (O), nitrogen (N), sulfur (S), silicon (Si), and selenium (Se). The heteroaryl rings typically comprise a four, five, six, seven, or eight membered aromatic ring, which may however be bonded to additional rings, so as to form a polycyclic aromatic ring system. At least one of the rings present in the ring system is aromatic and contains at least one ring.
heteroatom. Polycyclic heteroaryl groups include those having two or more heteroaryl rings fused together, as well as those having at least one monocyclic heteroaryl ring fused to one or more aromatic carbocyclic rings, non-aromatic carbocyclic rings, and/or non-aromatic cycloheteroalkyl rings. A heteroaryl group, as a whole, can have, for example, 5 to 24 ring atoms and contain 1-5 ring heteroatoms (i.e., 5-20 membered heteroaryl group). The heteroaryl group can be attached to the defined chemical structure at any heteroatom or carbon atom that results in a stable structure. Generally, heteroaryl rings do not contain O-O, S-S, or S-O bonds. However, one or more N or S atoms in a heteroaryl group can be oxidized (e.g., pyridine N-oxide, thiophene S-oxide, thiophene S,S-dioxide). Examples of heteroaryl moieties include furyl, fluorenyl, pyrrolyl, thienyl, oxazolyl, imidazolyl, thiazolyl, pyridyl, pyrimidinyl, quinazolinyl, quinolyl, isoquinolyl, and indolyl.

Alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, heterocycloalkyl, heterocycloalkenyl, aryl, and heteroaryl mentioned herein include both substituted and unsubstituted moieties, unless specified otherwise. Possible substituents on cycloalkyl, cycloalkenyl, heterocycloalkyl, heterocycloalkenyl, aryl, and heteroaryl include, but are not limited to, C1-C10 alkyl, C2-C10 alkenyl, C2-C10 alkynyl, C3-C20 cycloalkyl, C3-C20 cycloalkenyl, C1-C20 heterocycloalkyl, C1-C20 heterocycloalkenyl, C1-C10 alkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, amino, C1-C10 alkylamino, C1-C20 dialkylamino, arylamino, diarylamino, C1-C10 alkylsulfonamino, arylsulfonamino, C1-C10 alkylimino, arylimino, C1-C10 alkylsulfonimino, arylsulfonimino, hydroxyl, halo, thio, C1-C10 alkylthio, arylthio, C1-C10 alkylsulfonyl, arylsulfonyl, acylamino, aminoacetyl, aminothioacetyl, amidino, guanidine, ureido, cyano, nitro, nitroso, azido, acyl, thioacyl, acyloxy, carboxyl, and carboxylic ester. Cycloalkyl, cycloalkenyl, heterocycloalkyl, heterocycloalkenyl, aryl, and heteroaryl also include those fused with one or more additional rings.

In one embodiment the aryl group is optionally substituted with one or more of a halogen, or CF3. Examples of suitable heteroaryl groups include perfluorophenyl, pyridyl, piperidyl, or pyrrolyl. R4 represents a hydrogen, or hydroxyl. Optionally, R4 represents hydroxyl when A is a single bond. R5 represents a CmH2m+1 straight-chain or branched
alkyl, C$_2$-C$_{20}$-alkenyl, C$_2$-C$_{20}$-alkynyl, or C$_1$-C$_{20}$-alkoxy; m is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20. Bond A is a single bond or a double bond. Optionally, R$^4$ represents hydroxyl when A is a single bond. When the bond denoted as “A” is a double bond, both E and Z configurations have been contemplated. The term “alkenyl” refers to a linear or branched hydrocarbon moiety that contains at least one double bond, such as –CH=CH–CH$_3$. The term “alkynyl” refers to a linear or branched hydrocarbon moiety that contains at least one triple bond, such as –C≡C–CH$_3$.

When R$_2$ and R$_3$ are hydrogen, R$_1$ is not methyl.

When A is a trans-double bond, and when the configuration at C-2 is S and when the configuration at C-3 is R, R$_1$ is not hydroxymethyl (CH$_2$OH).

In another embodiment, with the proviso that when A is a double bond, R$_1$ is not hydroxymethyl.

In another preferred embodiment, the present invention provides a compound of formula I having the following structure:

Scheme A

In another preferred embodiment, the present invention provides a compound of formula I having the following structure:
Scheme B

\[
\text{OH} \quad \text{C}_{13}\text{H}_{27} \quad \text{OH} \\
\text{NH} \quad \text{X} \quad \text{NH} \\
\text{H} \quad \text{F} \\
\text{OH} \quad \text{C}_{13}\text{H}_{31} \\
\text{NH} \quad \text{X} \quad \text{NH} \\
\text{H} \\
\text{OH} \quad \text{C}_{14}\text{H}_{29} \\
\text{NH} \quad \text{X} \quad \text{NH} \\
\text{OH} \\
\text{CF}_3 \]

, , and


In another aspect, the invention provides a compound having formula II:

(II)

in which R\(^1\) is C\(_3\)-C\(_{12}\) alkyl, aryl, cycloalkyl, heterocyclyl, or heteroaryl, having a single cyclic ring or multiple condensed rings, quaternary ammonium group, or

in which Z is –OH, F, Br, Cl, or I. Optionally, the azido group (N\(_3\)) may be converted to a triazole-containing group. The triazole group may be substituted or non-substituted.

Examples of suitable heterocyclyl groups include piperidyl, pyrrolidyl, pyridyl, pyrrolyl, pyrimidinyl, furyl, imidazolyl, tetrazolyl, thienyl, thiazolyl, isothiazolyl, oxazolyl, isoxazolyl, triazolyl, quinolinyl, and isoquinolinyl, or an acyclic nitrogen-containing group.
In some embodiments, heteroaryl groups can be, for example, the 5- or 6-membered monocyclic and 5- or 6-membered bicyclic ring.

Examples of suitable $R^1$ groups are shown below:
in which \( n \) is 0, 1, 2, 3, 4, or 5; and \( R \) is \( C_3-C_7 \)-alkyl.

When \( n \) is 0, the adjacent atoms are connected by a single bond.
R\textsuperscript{2} is straight-chain or branched alkyl C\textsubscript{m}H\textsubscript{2m+1}, C\textsubscript{2}-C\textsubscript{20}-alkenyl, C\textsubscript{2}-C\textsubscript{20}-alkynyl, C\textsubscript{1}-C\textsubscript{20}-alkoxy, or C\textsubscript{2}-C\textsubscript{20}-alkyl-substituted heterocycle group. In some embodiments, the heterocyclic group can be triazole, oxadiazole, oxazole, or thiazole; m is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20; n is 0, 1, 2, 3, 4, or 5; when n is 0, the adjacent atoms are linked by a single bond. In some embodiments, the heterocyclic group can be triazole, oxadiazole, oxazole, or thiazole. W is –CH\textsubscript{2} or oxygen. X and Y are independently hydrogen, C\textsubscript{1}-C\textsubscript{4}-alkyl, or X and Y taken together are oxygen or sulfur.

The following compound is exemplary of an alkyl-substituted triazolium:

![Image of RB-065](image)

The triazole ring may be replaced by one of the following rings:

![Images of various rings](images)

In another preferred embodiment, the present invention provides a compound of formula II having the following structure:
Scheme C

5

10
and

, in which R is hydrogen or hydroxyl; m is 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15; and n is 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.
In another preferred embodiment, the present invention provides a compound of formula II having the following structure:

Scheme D

in which R is a \( C_nH_{2m+1} \) straight-chain or branched alkyl, \( C_2-C_{20} \)-alkenyl, \( C_2-C_{20} \)-alkynyl, or \( C_1-C_{20} \)-alkoxy; \( m \) is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20; \( X \) is \(-\mathrm{OH}, \ F, \ Br, \ Cl, \) or \( I \); \( n \) is 0, 1, 2, 3, 4, 5, or 6; and \( W \) is oxygen or carbon. When \( n \) is 0, the adjacent atoms are connected by a single bond. Optionally, the azido group \((N_3)\) may be converted to a triazole-containing group. The triazole group may be substituted or non-substituted.

In another aspect, the present invention provides for a compound having formula III:

(III)
in which $R^1$ is a $C_{m}H_{2m+1}$ straight-chain or branched alkyl, $C_{2}-C_{20}$-alkenyl, $C_{2}-C_{20}$-alkynyl, or $C_{1}-C_{20}$-alkoxy; $m$ is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20; $R^2$ is hydrogen, hydroxyl, or $C_{1}-C_{20}$-alkoxy; $R^3$ is oxygen or sulfur; $R^4$ is aryl or heteroaryl; and $X$ and $Y$ are independently NH or oxygen.

In another preferred embodiment, the present invention provides a compound of formula III having the following structure:

Scheme E

In yet another aspect, the present invention provides a method of selectively inhibiting SK1 in a cell by administering a compound of formula IV:

(IV)
The above-described compounds may be synthesized by any known method. Examples of synthesis schemes are provided in the examples below. The synthetic methods described herein may additionally include steps, either before or after the steps described specifically herein, to add or remove suitable protecting groups, or to introduce additional substituent groups in order to ultimately allow synthesis of the compounds disclosed herein.

This invention provides a method of inhibiting SK by administering the compounds described above. Accordingly, these compounds may be used to treat cells having elevated levels of S1P, and may therefore have therapeutic effect. Without wishing to be bound by theory, it is thought that cancer cells and pulmonary smooth muscle cells of people having pulmonary arterial hypertension have abnormally elevated levels of S1P. Therefore, these compounds can be used as anti-proliferative and pro-apoptotic/autophagic agents, and therefore have promise in the treatment of pulmonary arterial hypertension and cancer. In addition to blocking the enzymatic activity of SK, some of the compounds in the present invention stimulate the degradation of one of the isoforms of SK (SK1) by the proteasome of cancer and pulmonary smooth muscle cells. Some of the compounds in the invention inhibit or activate one of the two isoforms of SK and not the other isoform; these compounds can be used to analyze the roles of the two isoforms of SK, which are called SK1 and SK2. The isoform-selective SK inhibitors in the present disclosure are useful in establishing the role of SK1 and SK2 in cancer and vascular biology. In addition, the compounds of this invention that induce allosteric activation of SK1 are of use in treatment of fibrosis. Until the present invention, nothing was known about the allosteric effects of chiral derivatives of FTY720 on the allosteric activation or allosteric inhibition of SK1.

In another aspect, the present invention provides a method to selectively inhibit SK1 or SK2.
Selectively inhibits means that one isoform is inhibited more than the other isoform.

In another aspect, the present invention provides a method of selectively inhibiting SK1 by administering compounds described in Scheme A, Scheme C, or Scheme E to a cell.

In another aspect, the present invention provides a method of selectively inhibiting SK2 by administering compounds described in Scheme B to a cell.

In another aspect, the present invention provides a method of selectively activating SK1 by administering compounds described in Scheme D to a cell.

In another aspect, the present invention provides a method of inducing apoptosis in a cell by administering compounds described in Scheme A, Scheme C, or Scheme E to a cell.

Also provided in this invention are pharmaceutically acceptable derivatives, including salts (including amine salts, salts of mineral acids including but not limited to hydrochloride salts, phosphate-containing salts, and sulfate salts, salts of organic acids, and various alkali and alkali earth metal salts), esters, solvates, hydrates, and prodrugs of the compounds described herein. The term “prodrug” is intended to include any covalently bonded carriers of the disclosed compounds, which release the active compound on metabolism when the compound is administered to a living mammalian organism.

The compounds described herein may contain one or more chiral centers, in which case the compounds may exist as stereoisomers. These structures include all stereoisomers. Accordingly, the chemical structures depicted herein encompass all of the possible stereoisomeric forms, including the stereochemically pure form and stereoisomeric mixtures, which may be resolved using routine methods.

Also within the scope of this disclosure is a pharmaceutical composition containing an effective amount of at least one compound described herein and a pharmaceutical acceptable carrier. Further, this disclosure includes a method of administering an effective amount of one or more of the compound described herein to a
patient having a disease (e.g., an inherited or acquired disease). Examples of diseases that can be treated by the compounds disclosed above include hyper-proliferative diseases, such as cancer and vascular remodeling in pulmonary arterial hypertension.

“An effective amount” refers to the amount of an active compound described herein that is required to confer a therapeutic effect on the treated subject. Effective doses will vary, as recognized by those skilled in the art, depending on the types of diseases treated, route of administration, excipient usage, and the possibility of co-usage with other therapeutic treatment.

To practice the pharmaceutical composition described, a composition having one or more compound described herein can be administered parenterally, orally, nasally, rectally, topically, or buccally. The term “parenteral” as used herein refers to subcutaneous, intracutaneous, intravenous, intramuscular, intraarticular, intraarterial, intrasynovial, intrasternal, intrathecal, intralesional, or intracranial injection, as well as any suitable infusion technique.

A sterile injectable composition can be a solution or suspension in a non-toxic parenterally acceptable diluent or solvent, such as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that can be employed are mannitol, water, Ringer’s solution, and isotonic sodium chloride solution. In addition, fixed oils are conventionally employed as a solvent or suspending medium (e.g., synthetic mono- or diglycerides). Fatty acid, such as oleic acid and its glyceride derivatives are useful in the preparation of injectables, as are natural pharmaceutically acceptable oils, such as olive oil or castor oil, especially in their polyoxyethylated versions. These oil solutions or suspensions can also contain a long chain alcohol diluent or dispersant, carboxymethyl cellulose, or similar dispersing agents. Other commonly used surfactants such as Tweens or Spans or other similar emulsifying agents or bioavailability enhancers which are commonly used in the manufacture of pharmaceutically acceptable solid, liquid, or other dosage forms can also be used for the purpose of formulation.

A composition for oral administration can be any orally acceptable dosage form including capsules, tablets, emulsions and aqueous suspensions, dispersions, and solutions. In the case of tablets, commonly used carriers include lactose and corn starch.
Lubricating agents, such as magnesium stearate, are also typically added. For oral administration in a capsule form, useful diluents include lactose and dried corn starch. When aqueous suspensions or emulsions are administered orally, the active ingredient can be suspended or dissolved in an oily phase combined with emulsifying or suspending agents. If desired, certain sweetening, flavoring, or coloring agents can be added.

A nasal aerosol or inhalation composition can be prepared according to techniques well known in the art of pharmaceutical formulation. For example, such a composition can be prepared as a solution in saline, employing benzyl alcohol or other suitable preservatives, absorption promoters to enhance bioavailability, fluorocarbons, and/or other solubilizing or dispersing agents known in the art.

A composition having one or more active compound described herein can also be administered in the form of suppositories for rectal administration.

The carrier in the pharmaceutical composition must be “acceptable” in the sense that it is compatible with the active ingredient of the composition (and preferably, capable of stabilizing the active ingredient) and not deleterious to the subject to be treated. One or more solubilizing agents can be utilized as pharmaceutical excipients for delivery of an active compound described herein. Examples of other carriers include colloidal silicon oxide, magnesium stearate, cellulose, sodium lauryl sulfate, and D&C Yellow # 10.

The compound described herein can be preliminarily screened for their efficacy in treating an above-described disease by an in vitro assay and then confirmed by animal experiments and clinic trials. Other methods will also be apparent to those of ordinary skill in the art.

In this specification, groups of various parameters containing multiple members are described. Within a group of parameters, each member may be combined with any one or more of the other members to make additional sub-groups. For example, if the members of a group are a, b, c, d, and e, additional sub-groups specifically contemplated include any one, two, three, or four of the members, e.g., a and c; a, d, and e; b, c, d, and e; etc.
EXAMPLES

The following examples are illustrative and not intended to be limiting.

5 General Experimental Considerations

Synthetic Procedures

General experimental methods. All chemicals were reagent grade and were used as purchased. Reactions were carried out under a dry nitrogen atmosphere using oven-dried glassware and magnetic stirring. The solvents were dried as follows: THF was heated at reflux over sodium benzophenone ketyl; toluene was heated at reflux over sodium; CH₂Cl₂ were dried over CaH₂. The progress of the reactions was monitored by thin-layer chromatography analysis using aluminum-backed silica gel 60 F254 plates of 0.2-mm thickness. The spots were visualized with short wavelength ultraviolet light or by charring after spraying with 15% H₂SO₄. Flash chromatography was performed on silica gel grade 60 (230–400 ASTM mesh). ¹H NMR and ¹³C NMR spectra were recorded in δ units relative to deuterated solvents (CDCl₃ δ = 7.26 ppm for ¹H NMR and 77.00 ppm for ¹³C NMR); CD₃OD δ = 4.78, 3.31 ppm for ¹H NMR and 49.1 ppm for ¹³C NMR), which served as an internal reference, at 400 or 500 (for ¹H NMR) and 100 MHz (for ¹³C NMR), respectively. The purity of the products was >95% based on proton NMR spectra.

High-resolution mass spectra (HRMS) were recorded on an Agilent Technologies G6520A Q-TOF mass spectrometer using electrospray ionization (ESI). Optical rotations were recorded on a digital polarimeter at the sodium-D line at rt.

Example 1

Enzymatic Activity – Inhibition of SK1 by RB-005 and Its Analogues

Previous work has established SK1 and SK2 assays using sphingosine/[³²P]-ATP with over-expressed recombinant SK1 in HEK 293 cell lysates or purified SK1 or SK2

Select compounds of the invention are listed in Table 1.


<table>
<thead>
<tr>
<th>Compound</th>
<th>r</th>
<th>Compound</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>RB-001</td>
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<td>RB-11</td>
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<td>RB-002</td>
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<td>RB-12</td>
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<td>RB-003</td>
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<td>RB-13</td>
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<td>RB-004</td>
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<td>RB-006</td>
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<td>RB-021</td>
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<td>RB-022</td>
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</table>

Table 1. Structures of RB-001-RB-020
By using sphingosine concentrations of 3 µM and 10 µM, which correspond to the $K_m$ values of SK1 and SK2, respectively, the results of these assays demonstrate that RB-001–003, RB-005, RB-007–009, RB-019, and RB-021 (Table 1) are selective inhibitors of SK1 over SK2. SK1 activity was measured using 3 µM sphingosine and 250 µM ATP. SK2 activity was assayed using 10 µM sphingosine and 250 µM ATP ($n = 3$ for each compound); results expressed as % control ± S.D. The control is 100% and equals activity against sphingosine alone.

Small changes in the structure of this tertiary amine result in large changes in selectivity, arguing for the hypothesis that RB-005 is a selective inhibitor of SK1 (Fig. 1). To investigate the role of the hydroxyl group in the heterocyclic ring in the inhibition of SK1, the 4-hydroxy group was replaced with a 4-methyl group to afford RB-004, which is a moderate inhibitor of both SK1 and SK2 (Fig. 1); the 3-hydroxy regioisomer RB-019 is a selective but less potent SK1 inhibitor. To investigate the effect of the size of the heterocycle and the charge on the ring, heterocyclic amines RB-003 - RB-006 and quaternary ammonium compounds RB-013 - RB-016 were prepared and assayed. Fig. 1 shows that these modifications reduced inhibition of both SK1 and SK2. Relocation of the quaternary nitrogen functionality to an exocyclic position as in RB-016 - RB-018 also afforded nonselective SK inhibitors (Fig. 1). The data in Fig. 1 show that RB-005 has the highest selectivity for SK1 over SK2 (15-fold) and Fig. 2A shows that RB-005 exhibits an $IC_{50} = 3.6 ± 0.38$ µM for SK1.

Example 2

Examination of RB-005 Analogues as Possible SK Substrates

Furthermore, the possibility that the six inhibitors that bear a hydroxyl group may also serve as SK substrates was examined. At 50 µM, none of the compounds with the exception of RB-020 is a substrate for SK1 or SK2 (Table 2).
Table 2. RB-020 (50 \( \mu \)M) is a substrate of SK1 and SK2 and an inhibitor of SK1 activity against sphingosine (Sph). Sph was used at 3 and 10 \( \mu \)M for SK1 and SK2, respectively. Results are represented as % control \( \pm \) SD (n = 3) of control.

<table>
<thead>
<tr>
<th></th>
<th>SK1 activity (% control)</th>
<th>SK2 activity (% control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sph</td>
<td>100 ( \pm ) 4.4</td>
<td>100 ( \pm ) 12.8</td>
</tr>
<tr>
<td>Sph + RB-020</td>
<td>34.4 ( \pm ) 4.1</td>
<td>315 ( \pm ) 5.1</td>
</tr>
<tr>
<td>RB-020</td>
<td>27.5 ( \pm ) 3.0</td>
<td>228 ( \pm ) 17.9</td>
</tr>
</tbody>
</table>

RB-020 is less efficiently phosphorylated by SK1 than sphingosine, and probably overlaps the catalytic site of SK1 to inhibit phosphorylation of sphingosine (Table 2). However, this is not the case for SK2 where phosphorylation of sphingosine and RB-20 appear mutually exclusive (Table 2). RB-019 is a very weak substrate for SK2 (10% of the activity against sphingosine, data not shown) and inhibits SK1 activity with sphingosine as the substrate (Fig. 1). Both RB-019 and RB-020 contain a hydroxyl group that is likely to be phosphorylated by SK1 and SK2 (Table 2). It is noteworthy that RB-020 has a primary hydroxyl group attached to the heterocyclic ring through a 4-CH\(_2\) group, while RB-019 has a secondary hydroxyl group directly attached to C-3 of the heterocyclic ring; therefore, the latter hydroxyl group may be too far removed from the catalytic determinants of SK1 to be phosphorylated, but probably overlaps the substrate binding site to inhibit SK1 activity. RB-008 and RB-009 have a second nitrogen atom in the heterocyclic ring, and also possess a \(-(CH_2)_2OH\) and \(-(CH_2)_3OH\) group, respectively. However, RB-008 and RB-009 are not substrates for SK1 or SK2 (data not shown) but are inhibitors of SK1 (Fig. 1).

**Example 3**

**Enzymatic Activity – Inhibition of SK1 by RB-023 – RB-065**
<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>Compound</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>RB-023</td>
<td>$n = 1$</td>
<td>RB-032</td>
<td>$R_1 = \text{NH}_2$, $R_2 = \text{C}<em>9\text{H}</em>{17}$</td>
</tr>
<tr>
<td>RB-024</td>
<td>$n = 3$</td>
<td>RB-033</td>
<td>$R_1 = \text{NH}<em>2$, $R_2 = \text{C}</em>{12}\text{H}_{25}$</td>
</tr>
<tr>
<td>RB-025</td>
<td>$n = 4$</td>
<td>RB-034</td>
<td>$R_1 = \text{F}$, $R_2 = \text{C}<em>9\text{H}</em>{17}$</td>
</tr>
<tr>
<td>RB-026</td>
<td>$R_1 = \text{OH}$, $R_2 = \text{CH}_3$</td>
<td>RB-035</td>
<td></td>
</tr>
</tbody>
</table>
To investigate the structural determinants that result in selective SK1 inhibition, a series of analogues bearing a 4-hydroxypiperidinyl group was prepared in which the linker length between the aryl group and the piperidyl ring was varied. The effect of linker length on potency was assessed by comparing the % inhibition of SK1 and SK2 obtained with RB-023 (which has a one-carbon tether), RB-024 (three-carbon tether), and RB-025 (four-carbon tether). The linker length did not significantly alter the ability of RB-023, RB-024, and RB-025 to inhibit SK1 activity (Fig. 3). RB-023 – RB-025 also retained selectivity for SK1 over SK2 (Fig. 3).

The aliphatic chain at the \textit{para} position of the benzene ring of FTY720 is C$_8$H$_{17}$, which is known to be optimal for the action of FTY720 on its targets such as S1P receptors. To examine the role of the alkyl substituent on the benzene ring of RB-005, and thus the lipophilicity of the molecule, the inhibitory activities of RB-026 (which has a methyl group as the alkyl substituent), RB-027 (which has a $n$-hexyl group), RB-005 (which has a $n$-octyl group), and RB-028 (which has a $n$-dodecyl group) were examined. SK1 inhibition was decreased by more than 6-fold in RB-026 compared with RB-023 (Fig. 3). The almost complete lack of inhibition displayed by RB-026 against SK1 indicates that a larger alkyl group than a methyl group is required for inhibitory activity.
The importance of the 4-hydroxyl group of RB-005 was examined by replacing it with an azido, amino, fluoro, keto, or methoxy group (RB-029 – RB-036). Azido replacement (RB-029, RB-030) reduced SK1 inhibition markedly, while replacement of the 4-hydroxyl group with an amino group (RB-032) diminished the potency of SK1 inhibition. RB-032 inhibited SK1 activity with an IC₅₀ = 16.9 ± 1.6 µM (Fig. 2B). The isoform selectivity of SK1 over SK2 was retained for RB-032 (Fig. 3), suggesting that the amino group replacement maintains efficient binding to SK1.

Replacement of the 4-hydroxyl group of RB-005 with a fluoro (RB-034) or methoxy group (RB-036) eliminated inhibitory activity against SK1, while replacement with a keto group to produce RB-035 increased inhibition of SK2 and maintained inhibition of SK1 but eliminated the isoform selectivity (Fig. 3).

To investigate the role of the piperidyl group in inhibition of SK, experiments were carried out in which the piperidyl group was replaced with a pyrrolidine ring; the hydroxyl-containing substituent was retained (as either a chiral hydroxyl or a chiral hydroxymethyl group) but its orientation was varied, as shown in compounds RB-037 – RB-043. RB-037 and RB-038 retained inhibitory activity against SK1 despite having opposite configurations at C-3 of the pyrrolidin-3-ol group. Stereoisomers RB-040 and RB-042, which differ in the length of the aliphatic chain (C₈H₁₇ vs. C₁₂H₂₅) but possess the R configuration at C-2 of the 2-hydroxymethyl pyrrolidinyl group, were equipotent inhibitors of SK1 and SK2. RB-040 inhibits SK1 activity with an IC₅₀ = 2.2 µM, and SK2 with an IC₅₀ = 5.2 ± 0.82 µM (Fig. 2C, D). RB-042 inhibits SK1 activity with an IC₅₀ = 5.3 ± 0.5 µM and SK2 with an IC₅₀ = 5.0 ± 1.3 µM (Fig. 2E, F). The corresponding S enantiomers RB-041 and RB-043 were much less active (Fig. 3).

Experiments were performed to test the concentration-dependence of RB-041 and RB-043 inhibition of SK1 and SK2 activity. At a higher concentration of each (100 µM, compared to the 50 µM concentration data shown in Fig. 3), the inhibition of SK1 and SK2 activity with RB-041 was 72.2 ± 5.9% and 45.7 ± 2.6%, respectively, whereas with RB-043 the inhibition of SK1 and SK2 activity was 49.9 ± 6.2% and 49.7 ± 7%, respectively. These findings indicate that RB-041 and RB-043 can inhibit SK1 and SK2,
but that the sensitivity of inhibition compared with RB-040 and RB-042 is considerably reduced.

To further examine the influence of the length of the alkyl substituent on the benzene ring on SK activity, the extent of SK inhibition afforded by pyrrolidine derivatives RB-039, RB-042, and RB-043 was assessed. The ability of the compound to inhibit SK1 is abolished in RB-039 and RB-043 (Fig. 3), which have a methyl and a \( n \)-dodecyl group in the lipophilic tail, respectively. Replacing the methylene linker between the aryl group and the heterocycle with a keto group produced the benzamide analogues RB-044 – RB-050. Inhibition of SK1 was effectively abolished (Fig. 3) in these analogues, and also in the pyridine derivatives (RB-048 and RB-051). A series of triazole analogues of RB-005 (RB-005 – RB-065) was prepared and tested for SK inhibitory ability. As shown in Fig. 3, RB-065 is a highly selective SK1 inhibitor, whereas the other ten triazole analogues, all of which lack the 4-hydroxypiperidyl group, were inactive.

**Example 4**

**Examination of RB-023 – RB-065 as Possible SK Substrates**

The \( S \) enantiomers RB-041 and RB-043 and RB-037 are substrates for SK2 (Fig. 4).

**Example 5**

**Enzymatic Activity – Inhibition of SK1 or SK2 Enzymatic Activity by Analogues of 1-Deoxysphinganine, Sphingosine Derivatives, Dihydrosphingosine Derivatives, Phytosphingosine Derivatives, and Pachastrissamine Derivatives**

The structures of the 1-deoxysphingoid bases 55-21, 55-22, 77-7, and 77-13; the thiourea- PHS derivatives 67-301, 67-306, 67-310, and the urea-PHS derivative 67-311; the thiourea-sphinganine bases F01 and F02; and the thiourea-pachastrissamine derivative 67-341 are shown. Also displayed is the structures of the 4-sphingenine (sphingosine) adducts 67-320 and 67-330.
The effects of fluorine and trifluoromethyl substitution in the benzene ring of these putative inhibitors were examined. Although the \( p \)-fluorophenyl thiourea-PHS derivative 67-301 is a weak and nonselective SK inhibitor, the activity is improved by insertion of five fluorine atoms into the benzene ring to afford 67-306, which shows a moderate selectivity for inhibition of SK1 (Fig. 5). Thiourea 67-310 and urea 67-311, which are both \( p \)-trifluoromethylphenyl PHS derivatives, are moderately effective SK2 inhibitors (64.5 ± 4.9% and 53.9 ± 0.9% inhibition at 50 µM, respectively, \( n = 3 \)). The \( p \)-fluorophenyl thiourea-sphingosine derivative 67-320 is a highly selective SK2 inhibitor (79.2 ± 1.9% inhibition at 50 µM, \( n = 3 \)) whereas its \( p \)-trifluoromethylphenyl analogue 67-330 is a weak inhibitor of both SK isoforms (Fig. 5). The 4-\( epi \)-pachastrissamine pentafluorophenyl thiourea derivative 67-341 is a selective SK1 inhibitor (64.7 ± 5.3% inhibition at 50 µM, \( n = 3 \)) (Fig. 5).

The sphinganine thiourea derivative F-02 is a highly selective SK2 inhibitor (80 ± 2% inhibition at 50 µM, \( n = 3 \)), but its analogue F-01 is less selective (Fig. 5). The dose-
dependent inhibition analysis revealed that F-02 inhibited SK2 activity with an IC$_{50}$ value of 21.8 ± 4.2 µM but only very weakly inhibited SK1 activity (with an IC$_{50}$ value of 69 ± 5.5 µM) (Fig. 2G, H).

1-Deoxysphinganine analogue 55-21 and its N,N-dimethyl derivative 55-22 are selective SK1 inhibitors (Fig. 5). 55-21 inhibited SK1 activity with an IC$_{50}$ value of 7.1 ± 0.75 µM and SK2 activity with an IC$_{50}$ value of 766 ± 133 µM (Fig. 2I, J), whereas 77-7 inhibited SK1 activity with an IC$_{50}$ value of 27.8 ± 3.2 µM and SK2 activity with an IC$_{50}$ value of 300 ± 62.3 µM (data not shown). Thus, insertion of an oxygen atom into the aliphatic chain afforded 77-7, which is also a selective SK1 inhibitor. However, the N,N,N-trimethylammonium salt 77-13 is a nonselective SK isoform inhibitor.

**Example 6**


The possibility that the compounds that bear a hydroxyl group may also serve as SK substrates was examined. At 50 µM, F01, 77-13, 67-341, and 67-302 are weak substrates of SK1 (Fig. 6), but probably overlap the sphingosine binding site in SK1, thereby inhibiting catalytic phosphorylation of sphingosine. At 50 µM, F02 and F01 were very weak substrates of SK2, but 67-302 (cis-sphingosine) was efficiently phosphorylated by SK2 (Fig. 6). None of the other compounds were substrates.

**Example 7**

Examination of Effect of 55-21 on Growth of Pulmonary Arterial Smooth Muscle Cells

Figure 7 shows a comparison of the effects of two known, highly potent SK1 inhibitors, PF-543 and VPC96091, with 55-21 on the growth of pulmonary arterial smooth muscle cells (PASMC). This comparison, which is based on [$^{3}$H]-thymidine incorporation into DNA in PASMC, shows that the highly potent PF-543 and VPC96091
compounds were ineffective in inhibiting the growth of PASMC, whereas the less potent compound 55-21 of this invention was effective. Thus, the compounds disclosed here, including but not limited to 55-21, which have a moderate potency on inhibition of SK1 enzymatic activity, may possess a more favorable profile than highly potent inhibitors in terms of selectively abrogating SK1 function without exhibiting ‘off-target’ effects on sphingosine/ceramide metabolizing enzymes. In this regard, 55-21 recapitulates siRNA knockdown and genetic studies in terms of reducing cell growth; thus 55-21 is expected to have utility in unraveling the functions of SK1 in hyperproliferative disorders.

Example 8
Examination of (S)-FTY720 Vinylphosphonate Analogues as Allosteric Activators of SK1

(S)-FTY720 vinylphosphonate (at 50 μM) inhibits SK1 activity by 62.7 ± 0.9% (n = 3) using 3 μM sphingosine (Sph) as the substrate (see: Liu, Z., MacRitchie, N., Pyne, S., Pyne, N.J. & Bittman, R. (2013) Synthesis of (S)-FTY720 vinylphosphonate analogues and evaluation of their potential as sphingosine kinase 1 inhibitors. Bioorganic Medicinal Chemistry 21, 2503-2510). (S)-FTY720 vinylphosphonate is an uncompetitive inhibitor (with sphingosine) of SK1 with a $K_{i0} = 14.5 ± 4.4$ μM. (S)-FTY720 vinylphosphonate, which is not a substrate for SK1, competes with ATP for the ATP-binding site, but flips to bind to an allosteric site when ATP binds to the catalytic site. Therefore, the $-\text{P(O)(OH)}_2$ group appears to be essential for binding of (S)-FTY720 vinylphosphonate to both the catalytic and allosteric sites. This invention shows that replacement of the amino group with an azido group ($N_3$) produces an azido-alcohol derivative that activates of SK1 at low micromolar concentrations (and is not a substrate,
data not shown) (Fig. 8). Moreover, changing the azido-alcohol to an azido-fluoro derivative also gave a compound that activates SK1 at low concentrations. Both of these compounds induced a 30-60% stimulation of SK1 activity at low concentrations (Fig. 8). The structural difference in these azide-containing compounds concern fluorine, which can only accept hydrogen bonds, and a hydroxyl group, which can both accept and donate hydrogen bonds. The azido group is critical in this activity; the amino-fluoro derivative does not affect SK1 activity.

At concentrations below 50 µM, the azido-alcohol compound stimulated SK1 activity with an EC\textsubscript{50} of 8.3 ± 3.0 µM, n = 3 (Fig. 2K), indicative of binding to an allosteric site. At concentrations above 50 µM, the % activation in response to this compound diminished markedly (Fig. 2K). This biphasic response suggests that the compound might bind to the catalytic site (or alter its conformation) to inhibit SK1 activity at these higher concentrations. Similar results were obtained with the azido-fluoride compound (EC\textsubscript{50} of 5.7 ± 5.5 µM, n = 3, data not shown). The biphasic curves found with both compounds indicate that the azido group and not the fluoro or hydroxyl group is responsible for this phenomenon.

**Example 9**
**Inducing the Proteasomal Degradation of SK1 in Pulmonary Arterial Smooth Muscle Cells with Compounds 55-21 and RB-005**

Without wishing to be bound by theory, it is believed that proteasomal degradation of SK1 in response to SKi (2-((p-hydroxyanilino)-4-((p-chlorophenyl)thiazole) reduces intracellular S1P and increases C22:0-ceramide levels in prostate cancer cells, thereby promoting apoptosis (see: Loveridge, C., Tonelli, F., Leclercq, T., Lim, K.G., Long, S., Berdyshev, E., Tate, R.J., Natarajan, V., Pitson, S.M., Pyne, N.J. & Pyne, S. (2010) The sphingosine kinase 1 inhibitor 2-((p-hydroxyanilino)-4-((p-chlorophenyl)thiazole induces proteosomal degradation of sphingosine kinase 1 in mammalian cells. *Journal of Biological Chemistry* 285, 38841-38852). Figure 9A shows that treatment of pulmonary arterial smooth muscle cells (PASMC) with the SK1-
selective inhibitor 55-21 (10 µM, 24 h) reduced the expression of SK1; this was reversed by pre-treatment of the cells with the proteasomal inhibitor MG132. In contrast, treatment of PASMC with the SK2-selective inhibitor F-02 was without effect on SK1 expression (Fig. 9B), suggesting that changes in the ceramide-sphingosine-S1P rheostat regulated by SK2 is not accessible to the proteasome and, therefore, does not regulate SK1 turnover. The ability of RB-005 to promote the proteasomal degradation of SK1 in cells was also examined. As shown in Fig. 9C, treatment of PASMC with RB-005 (10 µM, 24 h) reduced the expression of SK1. The effect of RB-005 on SK1 expression was reversed by pre-treatment of the cells with the proteasomal inhibitor MG132.

**Example 10**

**Synthesis of the compounds described above**

**Synthetic Procedures**

*General experimental methods.* All chemicals were reagent grade and were used as purchased. Reactions were carried out under a dry nitrogen atmosphere using oven-dried glassware and magnetic stirring. The solvents were dried as follows: THF was heated at reflux over sodium benzophenone ketyl; toluene was heated at reflux over sodium; CH$_2$Cl$_2$ were dried over CaH$_2$. The progress of the reactions was monitored by thin-layer chromatography analysis using aluminum-backed silica gel 60 F254 plates of 0.2-mm thickness. The spots were visualized with short wavelength ultraviolet light or by charring after spraying with 15% H$_2$SO$_4$. Flash chromatography was performed on silica gel grade 60 (230–400 ASTM mesh). $^1$H NMR and $^{13}$C NMR spectra were recorded in δ units relative to deuterated solvents (CDCl$_3$ δ = 7.26 ppm for $^1$H NMR and 77.00 ppm for $^{13}$C NMR); CD$_3$OD δ = 4.78, 3.31 ppm for $^1$H NMR and 49.1 ppm for $^{13}$C NMR), which served as an internal reference, at 400 or 500 (for $^1$H NMR) and 100 MHz (for $^{13}$C NMR), respectively. The purity of the products was >95% based on proton NMR spectra. High-resolution mass spectra (HRMS) were recorded on an Agilent Technologies
G6520A Q-TOF mass spectrometer using electrospray ionization (ESI). Optical rotations were recorded on a digital polarimeter at the sodium-D line at rt.

**Preparation of Compounds 55-21, 55-22, and 77-13**

Scheme 1 outlines the preparation of 1-deoxysphinganine analogues 55-21 and 55-22 via cyclic sulfate intermediates of (2S,3R)-2-azidosphinganine. Azidoester 1 was prepared by asymmetric dihydroxylation of ethyl hexadecenoate using AD-mix-β, followed by conversion to a cyclic sulfate intermediate and regioselective azidation with sodium azide in aqueous acetone. Reduction of ester 1 with sodium borohydride in THF/MeOH (100:1) gave 2-azido-1,3-diol 2, which was converted to the cyclic sulfate intermediate 3 by reaction with SOCl2 in the presence of pyridine followed by oxidation of resulting cyclic sulfite with catalytic RuO4. Without further purification, 3 was subjected to reduction with sodium borohydride in DMF in the presence of sodium iodide, which removed the primary hydroxyl group, affording 55-21 in 79% yield. The reaction of 55-21 with formaldehyde in the presence of NaBH3CN in MeOH furnished the N,N-dimethylamino derivative 55-22 in 82% yield. N-Methylation of 55-22 with methyl tosylate in THF gave the N,N,N-trimethylammonium salt, 77-13.
Scheme 1. Synthesis of 1-deoxysphingoid derivatives 55-21, 55-22, and 77-13 by removal of the primary hydroxyl group from sphinganine via cyclic sulfate chemistry. Reagents and conditions: (a) NaBH$_4$, THF, MeOH, -78 °C – rt; (b) SOCl$_2$, py, CH$_2$Cl$_2$, -78 °C – rt; (c) cat. RuCl$_3$·3H$_2$O, NaIO$_4$, MeCN/H$_2$O (5:1), rt, 2 h; (d) NaBH$_4$ (2 equiv.), NaI (1 equiv.), DMF, 0 °C – rt, then aq. HCl (79%); (e) CH$_2$O (10 equiv.), NaBH$_3$CN (11 equiv.), MeOH, 0 °C – rt (82%); (f) MeOTs-$p$, THF, rt, overnight (100%).

(2S,3R)-2-Azidoctadecane-1,3-diol (2). This compound was prepared by reduction of azidoester 1 with an excess of NaBH$_4$ in THF/MeOH (100:1); $^1$H NMR (CDCl$_3$) δ 0.88 (t, $J = 6.6$ Hz, 3H), 1.26 (m, 26H), 1.5-1.58 (m, 2H), 3.42 (q, $J = 5.2$ Hz, 1H), 3.90 (d, $J = 1.2$ Hz, 2H); $^{13}$C NMR (CDCl$_3$) δ 14.1, 22.7, 25.6, 29.3, 29.48, 29.52, 29.56, 29.62, 29.68, 31.9, 33.7, 62.5, 66.8, 72.1.

(2S,3R)-1-Deoxy-2-amino-3-octadecanol (55-21). To a solution of azide 2 (1.50 g, 4.58 mmol) in 50 mL of CH$_2$Cl$_2$ was added 430 µL (5.92 mmol) of SOCl$_2$, followed by 950 µL (11.7 mol) of pyridine at -78 °C. The reaction mixture was stirred at -78 °C for 2 h, then at rt for 2 h, and was filtered through a pad of silica gel, which was washed with hexane/EtOAc (10:1). The filtrate was concentrated to give a cyclic sulfite intermediate. To a solution of the cyclic sulfite in 25 mL of MeCN were added 1.30 g (6.07 mmol) of crystalline NaIO$_4$ and 30 mg (0.14 mmol) of RuCl$_3$·3H$_2$O in 5 mL of H$_2$O. The mixture was stirred at rt for 2 h, and then was diluted with 250 mL of Et$_2$O and washed with H$_2$O. The ether layer was dried over Na$_2$SO$_4$ and concentrated to give 2-azido-1,3-cyclic sulfate 3. To a solution of 3 in 25 mL of DMF were added 380 mg (10.0 mmol) of NaBH$_4$ and 760 mg (5.07 mmol) of NaI at 0 °C. The mixture was stirred for 48 h at rt, and then was diluted with 200 mL of Et$_2$O, treated with 100 mL of 1 M of aqueous HCl solution for 4 h, and neutralized with 5 M of aqueous NaOH solution. The organic layer was separated, dried over Na$_2$SO$_4$, and concentrated. The product was purified by column chromatography on silica gel (eluting with CHCl$_3$/MeOH/concd NH$_4$OH 130:25:4) to afford 1.04 g (79%) of 55-21. The product was dissolved in a minimum volume of CHCl$_3$ and passed through a Cameo filter to remove dissolved silica gel: [α]$_D$ +5.0 ($c$ 0.40, MeOH); $^1$H NMR (CDCl$_3$) δ 0.88 (t, 3H, $J = 6.4$ Hz), 1.01 (d, 2H, $J = 6.4$ Hz), 1.26 (m,
26H), 1.35 (m, 1H), 1.73 (br s, 3H), 2.98 (m, 1H), 3.44 (m, 1H); $^{13}$C NMR (CDCl$_3$) δ 14.1, 22.7, 26.5, 29.34, 29.60, 29.61, 29.64, 29.69, 29.8, 31.9, 33.8, 63.4, 70.8.

(2S,3R)-2-N,N-Dimethylamino-3-octadecanol (55-22). To a mixture of 55-21 (457 mg, 1.60 mmol) and paraformaldehyde (500 mg, 16.6 mmol) in 50 mL of MeOH was added NaBH$_3$CN (1.10 g, 17.5 mmol) at 0 °C. After the mixture was stirred at rt for 48 h, it was diluted with 200 mL of EtOAc and was washed with brine. The organic layer was dried over Na$_2$SO$_4$ and concentrated. The product was purified by column chromatography on silica gel (eluting with hexane/EtOAc 1:1) to afford 412 mg (82%) of compound 55-22: [α]$_D$ +4.9 (c 0.35, MeOH); $^1$H NMR (CDCl$_3$) δ 0.88 (t, 3H, $J$ = 6.8 Hz), 0.98 (d, 3H, $J$ = 6.8 Hz), 1.26 (m, 26H), 1.53 (m, 1H), 2.21 (m, 2H), 2.30 (s, 6H), 3.72 (m, 1H); $^{13}$C NMR (CDCl$_3$) δ 9.6, 14.1, 22.7, 26.5, 29.35, 29.61, 29.62, 29.65, 29.68, 29.8, 31.9, 33.8, 42.8, 63.6, 70.8.

(2S,3R)-N,N,N-Trimethyl-3-hydroxy-2-octadecanaminium p-toluenesulfonate (77-13). A mixture of 55-22 (81 mg, 0.26 mmol) and methyl p-toluenesulfonate (63 mg, 0.34 mmol) in 5 mL of THF was stirred overnight at rt. After the N-methylation reaction was completed, the mixture was diluted with 5 mL of hexane. Filtration provided 125 mg (100%) of compound 77-13: $^1$H NMR (CDCl$_3$) δ 0.88 (t, 3H, $J$ = 6.8 Hz), 0.98 (d, 3H, $J$ = 6.8 Hz), 1.26 (m, 26H), 1.53 (m, 1H), 2.21 (m, 2H), 2.30 (s, 9H), 3.72 (m, 1H); $^{13}$C NMR (CDCl$_3$) δ 9.6, 14.1, 22.7, 26.5, 29.35, 29.61, 29.62, 29.65, 29.68, 29.8, 31.9, 33.8, 42.8, 63.6, 70.8; HRMS (M$^+$) calcd for m/z C$_{21}$H$_{48}$NO$^+$ 328.3574, found 328.3579.

Preparation of 77-7. As shown in Scheme 2, the synthesis of oxyspisulosine analogue 77-7, which contains an oxygen atom in the aliphatic chain, started with rac-1-$O$-tetradecylglycerol (4) using the same strategy as described in the preparation of 55-21. Oxidative cleavage of vicinal diol 4 with NaIO$_4$ afforded aldehyde 5. Because the E/Z stereoselectivity of the Horner-Wadsworth-Emmons (HWE) reaction in organic solvents is influenced by an oxygen-containing group adjacent to the aldehyde’s formyl group, (E)-$\alpha$,$\beta$-unsaturated ester 6 was prepared by the HWE reaction of 5 with (EtO)$_2$P(O)CH$_2$CO$_2$Et in aqueous 2-propanol the presence of K$_2$CO$_3$, affording ester 6 with good E selectivity. This method has the advantage that the wet aldehyde 5 can be
subjected to the HWE reaction. Asymmetric dihydroxylation of ester 6 with AD-mix-β proceeded smoothly, affording the desired chiral 2,3-diol ester 7 in 89% yield. Conversion of diol 7 to cyclic sulfate intermediate 8, followed by regioselective azidation gave azidoester 9, and reduction of the ester functionality in 9 with NaBH₄ in THF/MeOH (100:1) gave 2-azido1,3-diol 10. An attempted removal of the primary hydroxyl group from 1,3-diol 10 by the same method as shown in Scheme 1 did not succeed; when the cyclic sulfate of 10 was reduced with NaBH₄ a mixture of primary and secondary alcohols was formed that was difficult to purify. Therefore, a route was devised involving a dibutylstannane intermediate to synthesize 77-7 (Scheme 2).

Monotosylation by reaction of 10 with dibutyltin oxide followed by treatment of 11 with p-tosyl chloride gave intermediate 12, which was converted to 77-6b in two steps and 66% overall yield from 10. In contrast to the reduction of 3, the azido group was not completely reduced even in DMF at elevated temperature. Therefore, catalytic hydrogenolysis was necessary to complete the reduction of azide 12.

Scheme 2. Synthesis of 1-deoxysphingoid derivative 77-7 by removal of the primary hydroxyl group from (2S,3R)-oxysphinganine 10 via dibutylstannane-mediated monotosylation of 1,3-diol followed by NaBH₄ reduction. Reagents and conditions: (a) NaIO₄, THF/H₂O, 0 °C – rt; (b) (EtO)₂P(O)CH₂CO₂Et, K₂CO₃, 2-PrOH/H₂O (1:1) , 0 °C
– rt, overnight; (c) AD-mix β, MeSO₂NH₂, t-BuOH/H₂O (1:1), rt; (d) SOCl₂, py, CH₂Cl₂, 0 °C; (e) cat. RuCl₃·3H₂O, NaIO₄, MeCN/H₂O (5:2); (f) NaN₃ (3 equiv), Me₂CO/H₂O (2:1), then Et₂O, aq. H₂SO₄; (g) NaBH₄, THF/MeOH (100:1), 0 °C – rt; (h) Bu₂SnO, toluene, reflux; (i) p-TsCl, CH₂Cl₂, 0 °C – rt; (j) NaBH₄, THF, 0 °C – rt; (k) Pd(OH)₂/C, MeOH, rt; (l) CH₂O, NaBH₄CN (12.5 equiv), MeOH, 0 °C – rt (80%).

**Ethyl 4-Tetradecyloxy-2(E)-butenonate (6).** To a solution of NaIO₄ (4.12 g, 19.2 mmol) in 25 mL of water was added a solution of 1-O-tetradecyl-rac-glycerol (4, 4.27 g, 14.8 mmol) in 25 mL of THF at 0 °C, followed by stirring at rt. After the oxidative glycol cleavage was completed (about 2 h at rt), the mixture was concentrated under reduced pressure in order to remove THF and the formaldehyde formed, providing aldehyde 5. To the residue of crude 5 was added a solution of triethyl phosphonoacetate (4.68 g, 20.9 mmol) in 50 mL of 2-propanol, followed by dropwise addition of a solution of K₂CO₃ (26.0 g, 187 mmol) in 50 mL of water at 0 °C. The reaction mixture was gradually warmed to rt. After the mixture was stirred overnight at rt, the olefination product 6 was extracted with Et₂O (3 x 50 mL). The combined organic layers were washed with brine, dried (Na₂SO₄), and concentrated. The product was purified by flash column chromatography on silica gel (elution with hexane/EtOAc 25:1) to give 4.40 g (91%) of compound 6: ¹H NMR (CDCl₃) δ 0.88 (t, J = 7.0 Hz, 3H), 1.25 (s, 22H), 1.31 (t, J = 7.2 Hz, 3H), 1.57-1.63 (m, 2H), 3.46 (t, J = 6.6 Hz, 2H), 4.13 (dd, J = 2.0, 4.3 Hz, 2H), 4.21 (q, J = 7.2 Hz, 2H), 6.08 (dt, J = 2.0, 15.7 Hz, 1H), 6.97 (dt, J = 4.3, 15.7 Hz, 1H); ¹³C NMR (CDCl₃) δ 14.1, 14.2, 22.7, 26.1, 29.3, 29.45, 29.56, 29.58, 29.63, 29.64, 29.66, 31.9, 60.3, 69.3, 71.2, 121.1, 144.7, 166.3; HRMS (M + H)⁺ calcd for m/z C₂₀H₃₉O₃⁺ 327.2894, found 327.2893.

**Ethyl (2R,3R)-4-Tetradecyloxy-2,3-dihydroxybutanonate (7).** After a solution of 14.0 g of AD-mix-β in 100 mL of t-BuOH/H₂O (1:1) was stirred vigorously at rt for 1 h, 950 mg (10.0 mmol) of MeSO₂NH₂ was added, and stirring was continued for an additional 10 min. After 3.27 g (10.0 mmol) of compound 6 was added, the mixture was allowed to warm to rt. After the α,β-unsaturated ester was completely consumed (TLC), the reaction was quenched by the addition of sodium sulfite (1.5 g, 14.6 mmol). The
product was extracted with EtOAc. The combined extracts were dried (Na$_2$SO$_4$) and concentrated. Chromatography on silica gel (elution with hexane/EtOAc 2:1) gave 3.07 g (89%) of compound 7: $[\alpha]_D^2 +7.8$ (c 1.61, CHCl$_3$/MeOH 1:1); $^1$H NMR (CDCl$_3$) $\delta$ 0.87 (t, $J = 6.6$ Hz, 3H), 1.24 (s, $J = 22$Hz, 1H), 1.31 (t, $J = 7.2$ Hz, 3H), 1.54-1.57 (m, 2H), 2.61 (d, $J = 7.4$ Hz, 1H), 3.27 (d, $J = 5.9$ Hz, 1H), 3.47 (t, $J = 6.7$ Hz, 2H), 3.51-3.62 (m, 2H), 4.06-4.13 (m, 1H), 4.23 (dd, $J = 2.1$, 5.8 Hz, 1H), 4.27 (q, $J = 7.2$ Hz, 2H); $^{13}$C NMR (CDCl$_3$) $\delta$ 14.08, 14.10, 22.7, 26.0, 29.3, 29.4, 29.53, 29.56, 29.58, 29.62, 29.63, 29.65, 31.9, 62.0, 70.8, 71.0, 71.2, 71.7, 173.1; HRMS (M + H)$^+$ calcd for $m/z$ C$_{20}$H$_{31}$O$_5$ $^+$ 361.2949, found 361.2952.

**Ethyl (2$S$,3$R$)-4-Tetradecyloxy-2-azido-3-hydroxybutanonate (9).** To a solution of 2.89 g (8.01 mmol) of compound 7 in 50 mL of CH$_2$Cl$_2$ was added 1.3 mL (16.1 mmol) of pyridine at rt. After the mixture was stirred and chilled to 0 °C, 760 $\mu$L (10.4 mmol) of SOCl$_2$ was added slowly. The reaction mixture was stirred for 30 min, and then was filtered through a pad of silica gel in a sintered glass funnel. The pad was washed with hexane/EtOAc 10:1, and the filtrate was concentrated and further dried under high vacuum (~1 torr) for 2 h. To a solution of the crude cyclic sulfite in 20 mL of MeCN was added 2.57 g (12.0 mmol) of NaIO$_4$. The heterogeneous mixture was stirred vigorously while a solution of 2.8 mg (0.080 mmol) of RuCl$_3$·3H$_2$O in 8 mL of H$_2$O was added. After the full consumption of the starting cyclic sulfite was observed (~1 h), the reaction mixture was diluted with 250 mL of Et$_2$O and washed with brine. The organic phase was dried (Na$_2$SO$_4$) and passed through a small pad of silica gel. Concentration of the filtrate gave crude cyclic sulfate 8, which was used without further purification. To a solution of 8 in 30 mL of acetone was added 1.56 g (24 mmol) of NaN$_3$, followed by 15 mL of H$_2$O. The reaction mixture was stirred at rt until 8 was fully consumed. After acetone and water were removed, the residue was dissolved in 100 mL of Et$_2$O, and the solution was treated with 50 mL of 20% aqueous H$_2$SO$_4$ in a fume hood with vigorous stirring of the heterogeneous mixture until the hydrolysis was completed. The layers were separated, and the aqueous layer was extracted with Et$_2$O (2 x 50 mL). The combined organic layers were treated with anhydrous K$_2$CO$_3$ (~100 mg) to remove the dissolved H$_2$SO$_4$ and then were dried (Na$_2$SO$_4$). After concentration, the product was purified by
chromatography on silica gel (elution with hexane/EtOAc 10:1) to give 2.44 g (79%) of azide 9: $[\alpha]_D +40.9$ (c 0.40, CHCl$_3$); $^1$H NMR (CDCl$_3$) $\delta$ 0.88 (t, $J = 6.7$ Hz, 3H), 1.26 (s, 22H), 1.33 (t, $J = 7.2$ Hz, 3H), 1.54-1.57 (m, 2H), 2.77 (d, $J = 7.4$ Hz, 1H), 3.41-3.51 (m, 2H), 3.52-3.61 (m, 2H), 4.03-4.13 (m, 2H), 4.28 (q, $J = 7.2$ Hz, 2H); $^{13}$C NMR (CDCl$_3$) $\delta$ 14.08, 14.10, 22.7, 26.0, 29.3, 29.4, 29.56, 29.59, 29.63, 29.67, 31.9, 62.0, 63.2, 70.3, 70.8, 71.8, 168.7; HRMS (M + Na)$^+$ calcd for $m/z$ C$_{20}$H$_{39}$N$_3$NaO$_4$ $^+$ 408.2833, found 408.2837.

$(2S,3R)$-4-Tetradecyloxy-2-azido-1,3-butanediol (10). To a solution of 2.43 g (6.30 mmol) of compound 9 in 100 mL of THF was added 380 mg (10.0 mmol) of NaBH$_4$, followed by 1 mL of MeOH at 0 °C. The reaction mixture was stirred vigorously while the reaction mixture was allowed to warm to rt until the disappearance of 9 (monitored by TLC). After THF and water were removed, the residue was dissolved in 200 mL of EtOAc, and then was washed with brine and water. The organic layer was dried (Na$_2$SO$_4$) and concentrated. The product was purified by column chromatography on silica gel (elution with hexane/EtOAc 10:1, 8:1, and 6:1) to give 1.11 g (51%) of 10: $[\alpha]_D +3.5$ (c 0.40, CHCl$_3$); $^1$H NMR (CDCl$_3$) $\delta$ 0.87 (t, $J = 6.7$ Hz, 3H), 1.26 (s, 22H), 1.53-1.63 (m, 2H), 2.51 (t, $J = 5.7$ Hz, 1H), 2.78 (d, $J = 6.0$ Hz, 1H), 3.40-3.65 (m, 5H), 3.77-3.87 (m, 2H), 3.88-3.97 (m, 1H); $^{13}$C NMR (CDCl$_3$) $\delta$ 14.1, 22.7, 26.0, 29.51, 29.56, 29.59, 29.63, 29.65, 29.67, 31.9, 62.7, 64.0, 70.7, 71.2, 71.8; HRMS (M + H)$^+$ calcd for $m/z$ C$_{20}$H$_{39}$O$_3$ $^+$ 327.2894, found 327.2893.

$(2S,3R)$-4-Tetradecyloxy-2-amino-3-butanol (77-6b). A mixture of 10 (382 mg, 1.11 mmol) and $n$-Bu$_2$SnO (280 mg, 1.12 mmol) in 25 mL of toluene was heated at reflux until a clear solution was formed. The solvent was removed under reduced pressure and the residue (11) was dried further under high vacuum for 2 h. After the dry residue was dissolved in 25 mL of CH$_2$Cl$_2$, 220 mg (1.15 mmol) of p-toluenesulfonyl chloride was added at 0 °C. After the mixture was stirred overnight at rt, the reaction was quenched by addition of H$_2$O (20 µL, 1.11 mmol). The mixture was filtered through a pad of Celite, which was washed with 100 mL of Et$_2$O. The filtrate was washed with brine, aqueous saturated NaHCO$_3$ solution, and water. The organic layer was dried (Na$_2$SO$_4$) and
concentrated to give crude tosylate 12. To a solution of 12 in 25 mL of THF was added 100 mg (2.64 mmol) of NaBH₄ at 0 °C. The mixture was stirred for 48 h at rt, diluted with 200 mL of Et₂O, and washed with brine. The ether layer was dried (Na₂SO₄) and concentrated. To the residue was added Pearlman’s catalyst (50 mg) in 25 mL of MeOH, and the mixture was stirred overnight at rt under a hydrogen atmosphere. After the catalyst was removed by filtration, the product was purified by column chromatography on silica gel (eluting with CHCl₃/MeOH/conc. NH₄OH 130:25:4) to afford 241 mg (66%) of 77-6b. The product was dissolved in a minimum volume of CHCl₃ and passed through a Cameo filter to remove dissolved silica gel: [α]D +4.5 (c 0.40, MeOH); ¹H NMR (CDCl₃) δ 0.88 (t, J = 6.4 Hz, 3H), 1.10 (d, J = 6.4 Hz, 2H), 1.26 (m, 26H), 1.57 (m, 2H), 2.45 (br s, 3H), 3.05 (m, 1H), 3.43-3.49 (m, 4H), 3.62 (m, 1H); ¹³C NMR (CDCl₃) δ 14.1, 18.6, 22.7, 26.1, 29.33, 29.44, 29.56, 29.58, 31.9, 48.8, 71.7, 71.8, 73.6; HRMS (M + H)⁺ calcd for m/z C₁₈H₄₀NO₂⁺ 302.3054, found 302.3057.

(2S,3R)-4-Tetradecyloxy-2-(N,N-dimethylamino)-3-butanol (77-7). To a mixture of paraformaldehyde (50 mg, 1.6 mmol) and 77-6b (43 mg, 0.14 mmol) in 10 mL of MeOH was added NaBH₃CN (110 mg, 1.75 mmol) at 0 °C. The mixture was stirred at rt for 48 h, and then was diluted with 200 mL of EtOAc and washed with brine. The organic layer was dried over Na₂SO₄ and concentrated. The product was purified by column chromatography on silica gel (eluting with CHCl₃, CHCl₃/MeOH 25:1, and then CHCl₃/MeOH/NH₄OH 130:25:4) to afford 38 mg (80%) of product: ¹H NMR (CDCl₃) δ 0.88 (t, 3H, J = 6.6 Hz), 1.02 (d, 3H, J = 6.7 Hz), 1.26 (m, 26H), 1.58 (q, J = 6.7 Hz, 2H), 2.27 (s, 6H), 2.49 (q, J = 6.6 Hz, 1H), 3.37 (dd, J = 7.9, 9.5 Hz, 1H), 3.46 (t, J = 6.6 Hz, 2H), 3.54 (dd, J = 3.6, 9.5 Hz, 1H), 3.87-3.82 (m, 1H); ¹³C NMR (CDCl₃) δ 8.5, 14.1, 22.7, 26.1, 29.35, 29.46, 29.59, 29.64, 29.67, 31.9, 41.7, 61.2, 70.8, 71.5, 73.1; HRMS (M + H)⁺ calcd for m/z C₂₀H₄₄NO₂⁺ 330.3367, found 330.3365.

General Procedure for Preparation of N-Aryliourea and N-Arylurea Derivatives. To a solution of the amino-sphingoid base (1.02 mmol) in 30 mL of CHCl₃/MeOH (1:1) was added the aryl isothiocyanate or aryl isocyanate (XC₆H₄NCS or XC₆H₄NCO, 1.00 mmol) at rt. The mixture was stirred overnight, and then was
concentrated under reduced pressure. The product was purified by column chromatography on silica gel.


**Scheme 3.** Synthesis of thiourea derivatives 67-320, 67-330, 67-301, 67-310, 67-311, 67-306, F-01, and F-02 by the reaction of sphingosine, D-ribo-phytosphingosine (PHS), or sphinganine with an aryl isothiocyanate or an aryl isocyanate.
Synthesis of 67-341.

Data for 67-341. Yield: 84%; $^1$H NMR (CDCl$_3$/CD$_3$OD) $\delta$ 0.88 (t, $J$ = 3.7 Hz, 3H), 1.26 (m, 24H), 1.41-1.49 (m, 2H), 3.62 (t, $J$ = 9.4 Hz, 1H), 3.73-3.78 (m, 1H), 4.00-4.05 (m, 1H), 4.22-4.34 (m, 1H), 4.77 (br s, 1H), 7.33 (s, 1H); $^{13}$C NMR (CDCl$_3$/CD$_3$OD) $\delta$ 13.9, 22.5, 25.6, 29.2, 29.42, 29.46, 29.52, 29.55, 31.8, 33.3, 56.7, 70.0, 74.0, 82.5, 85.2, 114.1, 136.4, 138.9, 141.7, 142.9, 145.3, 183.4; HRMS (M + H)$^+$ m/z calcd. for C$_{25}$H$_{38}$F$_5$N$_2$O$_2$S$^+$ 525.2569, found 525.2570.

Synthesis of cis-Sphingosine (67-302).

Data for carbamate 67-304. $^1$H NMR (CDCl$_3$) $\delta$ 0.88 (t, $J$ = 6.6 Hz, 3H), 1.26 (m, 20H), 1.31-1.40 (m, 2H), 1.98-2.14 (m, 2H), 3.64 (d, $J$ = 4.7 Hz, 2H), 3.86 (dt, $J$ = 4.7, 8.1 Hz, 1H), 5.46 (t, $J$ = 8.6 Hz, 1H), 5.57-5.63 (m, 1H), 5.75-5.82 (m, 1H), 6.64 (s, 1H); $^{13}$C NMR (CDCl$_3$) $\delta$ 14.1, 22.7, 27.9, 29.2, 29.32, 29.34, 29.42, 29.5, 29.64, 29.67, 31.9, 57.3, 61.6, 75.2, 122.0, 137.7, 160.6; HRMS (M + Na)$^+$ m/z calcd. for C$_{19}$H$_{35}$NNaO$_3$ 348.2509, found 348.2509.

Data for 67-302 (cis-Sphingosine). $^1$H NMR (CDCl$_3$/CD$_3$OD) $\delta$ 0.89 (t, $J$ = 7.0 Hz, 3H), 1.27 (m, 22H), 2.00-2.22 (m, 2H), 3.19-3.24 (m, 1H), 3.65-3.86 (m, 2H), 4.69-4.75 (m, 1H), 5.30-5.45 (m, 1H), 5.63-5.74 (m, 1H); $^{13}$C NMR (CDCl$_3$/CD$_3$OD) $\delta$ 13.5, 22.2, 27.4,
28.9, 29.08, 29.13, 29.18, 29.21, 29.24, 31.5, 67.1, 57.8, 64.5, 125.7, 135.2; HRMS (M + H)^+ m/z calcd. for C_{18}H_{38}NO_2^+ 300.2897, found 300.2899.

**Preparation of Compounds RB-001 through RB-010, and of RB-019, RB-020, and RB-011 through RB-018**

**General Procedure for Preparation of Tertiary Amine Analogues of FTY720.**

The tertiary amines shown in Table 1 were prepared in good yields by displacement of mesylate ion from 4-(octylphenethyl) methanesulfonate (prepared as displayed in Scheme 4) with amines in acetonitrile. Some of the quaternary ammonium salts were prepared by N-alkylation of the tertiary amines (and the secondary amine RB-006) with an excess of MeI and K_2CO_3 in acetonitrile whereas others (RB-011, RB-012, RB-017, and RB-018) were prepared by N-alkylation with tertiary N-methylamines.

![Scheme 4. Preparation of 4-octylphenethyl methanesulfonate.](image)

**4-Octanoylphenethyl Acetate.** To a suspension of AlCl_3 (1.2 g, 9.1 mmol) in 1,2-dichloroethane (DCE, 25 mL) was added dropwise caprylyl chloride (1.03 mL, 6.1 mmol). After the reaction mixture had stirred at rt for 1 h, a solution of phenethyl acetate (1.0 g, 6.1 mmol) in DCE (5 mL) was added. The mixture was stirred for 12 h at rt, poured into 1 N NaOH, and extracted with EtOAc. The extract was washed with brine, dried, and evaporated. Silica gel chromatography, eluting with hexanes/EtOAc (8:1), gave 4-octanoylphenethyl acetate (1.06 g, 60%) as a yellow waxy solid; \(^1\)H NMR (400
MHz, CDCl$_3$) $\delta$ 0.88 (t, $J$ = 6.9 Hz, 3H), 1.24–1.37 (m, 8H), 1.59–1.67 (m, 2H), 2.04 (s, 3H), 2.94 (td, $J$ = 7.4, 2.4 Hz, 2H), 3.00 (t, $J$ = 6.9 Hz, 2H), 4.27–4.32 (m, 2H), 7.30 (d, $J$ = 8.2 Hz, 2H), 7.91 (d, $J$ = 8.3 Hz, 2H).

4-Octylphenethyl Acetate. To a solution of 4-octanoylphenethyl acetate (1.0 g, 3.4 mmol) in trifluoroacetic acid (10 mL) was added triethylsilane (1.1 mL, 6.9 mmol). The reaction mixture was stirred at rt for 3 h, concentrated, and diluted with EtOAc. The solution was washed with 1 N NaOH and brine, dried, and evaporated. Silica gel chromatography, eluting with hexanes/EtOAc (15:1), gave the product (780 mg, 82%) as a yellow liquid; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 0.88 (t, $J$ = 6.8 Hz, 3H), 1.22–1.31 (m, 10H), 1.58–1.60 (m, 2H), 2.04 (s, 3H), 2.57 (td, $J$ = 7.6, 1.5 Hz, 2H), 2.88–2.93 (m, 2H), 4.24–4.29 (m, 2H), 7.11 (s, 4H).

4-Octylphenethyl Alcohol. To a solution of 4-octylphenethyl acetate (500 mg, 1.81 mmol) in MeOH (10 mL) was added sodium methoxide (195 mg, 3.61 mmol). The mixture was heated at reflux for 6 h, evaporated, and partitioned between EtOAc and water. The organic layer was separated and washed with brine. The solution was dried over Na$_2$SO$_4$ and evaporated to provide 4-octylphenethyl alcohol (390 mg, 92%) as a yellow oil; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 0.88 (t, $J$ = 6.8 Hz, 3H), 1.25–1.31 (m, 10H), 1.55–1.63 (m, 2H), 2.57 (t, $J$ = 7.8 Hz, 2H), 2.83 (t, $J$ = 6.6 Hz, 2H), 3.84 (t, $J$ = 6.6 Hz, 2H), 7.11 (s, 4H).

4-Octylphenethyl Methanesulfonate (Scheme 4).

![Methanesulfonate](image)

To a solution of 4-octylphenethyl alcohol (200 mg, 0.853 mmol) and triethylamine (1.19 mL, 8.53 mmol) in CH$_2$Cl$_2$ (8 mL) at 0 °C was added methanesulfonyl chloride (0.33 mL, 4.27 mmol). After being stirred at rt for 5 h, the reaction mixture was evaporated, diluted with water, and the product was extracted with EtOAc. The extract was washed with brine, dried, and evaporated. Purification by silica gel chromatography, eluting with hexanes/EtOAc (5:1), gave 253 mg (95%) of the
mesylate as a yellow liquid; $^1$H NMR (400 MHz, CDCl$_3$) δ 0.87 (t, $J$ = 6.9 Hz, 3H), 1.26–1.30 (m, 10H), 1.58 (m, 2H), 2.57 (t, $J$ = 7.7 Hz, 2H), 2.83 (s, 3H), 3.02 (t, $J$ = 7.0 Hz, 2H), 4.40 (t, $J$ = 7.0 Hz, 2H), 7.14 (s, 4H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 14.1, 22.7, 29.3 (2C), 29.5, 31.6, 31.9, 35.3, 35.6, 37.3, 70.6, 128.8, 128.9, 133.4, 141.9; ESI-HRMS (M + Na)$^+$ m/z calcd for C$_{17}$H$_{28}$NaO$_3$S 335.1657, found 335.1655.

Preparation of 1-(4-Octylphenethyl)pyrrolidine (RB-001)

![RB-001](image)

To a solution of 4-octylphenethyl methanesulfonate (15 mg, 0.040 mmol) in 3 mL of acetonitrile was added pyrrolidine (80 µL, 0.90 mmol). The reaction mixture was stirred at 50 °C for 24 h and concentrated. Purification by silica gel chromatography, eluting with CH$_2$Cl$_2$/MeOH (5:1), gave 11 mg (80%) of RB-001 as a slightly yellow waxy solid; $^1$H NMR (400 MHz, CDCl$_3$) δ 0.88 (t, $J$ = 6.8 Hz, 3H), 1.20–1.29 (m, 10H), 1.56–1.60 (m, 2H), 2.03–2.06 (m, 4H), 2.13–2.19 (m, 2H), 2.56 (t, $J$ = 7.8 Hz, 2H), 3.03–3.10 (m, 6H), 7.10–7.15 (m, 4H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 14.1, 22.7, 23.4, 29.3, 29.5, 31.0, 31.5, 31.9, 32.7, 35.6, 53.4, 53.9, 57.4, 128.5, 128.8, 134.3, 141.7; ESI-HRMS (M + H)$^+$ m/z calcd for C$_{20}$H$_{34}$N 288.2691, found 288.2689.

Preparation of 1-(4-Octylphenethyl)piperidine (RB-002)

![RB-002](image)

To a solution of 4-octylphenethyl methanesulfonate (100 mg, 0.32 mmol) in 5 mL of acetonitrile was added 4-hydroxypiperidine (162 mg, 1.60 mmol). The reaction mixture was stirred at 50 °C for 12 h and concentrated. Purification by silica gel chromatography, eluting with CH$_2$Cl$_2$/MeOH (5:1), gave 87 mg (86%) of RB-002 as a colorless oil; $^1$H NMR (400 MHz, CDCl$_3$) δ 0.87 (t, $J$ = 6.8 Hz, 3H), 1.23–1.29 (m, 10H), 1.54–1.60 (m, 4H), 1.77–1.80 (m, 4H), 2.56 (t, $J$ = 7.8 Hz, 2H), 2.71–2.78 (m, 6H), 2.91–2.95 (m, 2H), 7.09–7.14 (m, 4H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 14.1, 22.7, 23.6, 25.8,
The azepane derivative **RB-003** was prepared from 4-octylphenethyl methanesulfonate according to a coupling procedure similar to that described for compound **RB-001**, using hexamethyleneimine. Yield = 76%; \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 0.88 (t, \(J = 6.8\) Hz, 3H), 1.22–1.32 (m, 10H), 1.54–1.61 (m, 2H), 1.69–1.84 (m, 4H), 1.92–2.13 (m, 4H), 2.56 (t, \(J = 7.7\) Hz, 2H), 3.14–3.24 (m, 6H), 7.11–7.15 (m, 4H); \(^1^3\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 14.1, 22.7, 23.5, 26.9, 29.3, 29.4, 29.5, 30.3, 31.5, 31.9, 35.5, 54.5, 58.8, 128.6, 129.0, 133.3, 142.1; ESI-HRMS (M + H\(^+\)) \(^m/z\) calcd for C\(_{21}\)H\(_{36}\)N 302.2847, found 302.2842.

**Preparation of 4-Methyl-1-(4-octylphenethyl)piperidine (RB-004)**

Compound **RB-004** was prepared from 4-octylphenethyl methanesulfonate according to a coupling procedure similar to that described for compound **RB-001**, using 4-methylpiperidine. Yield = 73%; \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 0.87 (t, \(J = 6.8\) Hz, 3H), 0.99 (d, \(J = 6.2\) Hz, 3H), 1.25–1.32 (m, 10H), 1.52–1.66 (m, 5H), 1.74–1.77 (m, 2H), 2.31–2.36 (m, 2H), 2.56 (t, \(J = 7.8\) Hz, 2H), 2.81–2.85 (m, 2H), 2.98–3.02 (m, 2H), 3.25–3.28 (m, 2H), 7.11 (dd, \(J = 8.2, 10.6\) Hz, 4H); \(^1^3\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 14.1, 19.8, 21.3, 22.7, 25.5, 29.3, 29.4, 29.5, 31.5, 32.5, 35.6, 53.4, 128.6, 128.7, 132.7, 141.3; ESI-HRMS (M + H\(^+\)) \(^m/z\) calcd for C\(_{22}\)H\(_{38}\)N 316.3004, found 316.3003.

**Preparation of 1-(4-Octylphenethyl)piperidin-4-ol (RB-005)**
To a solution of 4-octylphenethyl methanesulfonate (30 mg, 0.096 mmol) in 5 mL of acetonitrile was added 4-hydroxypiperidine (49 mg, 0.48 mmol). The reaction mixture was stirred at 50 °C for 12 h and concentrated. Purification by silica gel chromatography, eluting with CH$_2$Cl$_2$/MeOH (5:1), gave 27 mg (90%) of RB-005 as a colorless oil; $^1$H NMR (400 MHz, CDCl$_3$) δ 0.87 (t, $J$ = 6.8 Hz, 3H), 1.22–1.30 (m, 10H), 1.55–1.68 (m, 6H), 1.94–1.97 (m, 2H), 2.24 (t, $J$ = 9.1 Hz, 2H), 2.54–2.61 (m, 4H), 2.76–2.80 (m, 2H), 2.84–2.89 (m, 2H), 3.71–3.75 (m, 1H), 7.08–7.12 (m, 4H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 14.1, 22.7, 29.3, 29.4, 29.5, 31.6, 31.9, 33.4, 34.4, 35.6, 51.0, 60.6, 128.4, 128.6, 137.4, 140.7; ESI-HRMS (M + H)$^+$ m/z calcd for C$_{21}$H$_{36}$NO 318.2797, found 318.2793.

Preparation of 2-(4-Octylphenyl)-N-((tetrahydrofuran-2-yl)methyl)ethanamine (RB-006)

The secondary amine derivative RB-006 was prepared from 4-octylphenethyl methanesulfonate according to a coupling procedure similar to that described for compound RB-001, using tetrahydrofurfurylamine. Yield = 87%; $^1$H NMR (400 MHz, CDCl$_3$) δ 0.89 (t, $J$ = 6.7 Hz, 3H), 1.26–1.39 (m, 10H), 1.49–1.60 (m, 4H), 1.84–1.91 (m, 2H), 2.56 (t, $J$ = 7.7 Hz, 2H), 2.68–2.78 (m, 2H), 2.81–2.90 (m, 2H), 2.94–2.97 (m, 2H), 3.73 (dd, $J$ = 7.0, 14.0 Hz, 1H), 3.83 (dd, $J$ = 7.0, 14.4 Hz, 1H), 4.02–4.06 (m, 1H), 7.11 (dd, $J$ = 8.3, 10.7 Hz, 4H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 14.1, 22.7, 25.7, 29.3, 29.4, 29.5, 29.7, 31.6, 31.9, 35.4, 35.6, 51.3, 53.9, 128.5, 128.6, 136.6, 140.9; ESI-HRMS (M + H)$^+$ m/z calcd for C$_{21}$H$_{36}$NO 318.2797, found 318.2795.
Preparation of 1-(4-Octylphenethyl)piperazine (RB-007)

Compound RB-007 was prepared from 4-octylphenethyl methanesulfonate according to a coupling procedure similar to that described for compound RB-001, using piperazine. Yield = 72%; $^1$H NMR (400 MHz, CDCl$_3$) δ 0.87 (t, $J = 6.8$ Hz, 3H), 1.23–1.32 (m, 10H), 1.55–1.60 (m, 2H), 2.49–2.64 (m, 8H), 2.75–2.79 (m, 2H), 3.01 (t, $J = 4.6$ Hz, 4H), 7.07–7.12 (m, 4H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 14.1, 22.7, 29.3, 29.4, 29.5, 31.6, 31.9, 32.9, 35.6, 45.2, 53.1, 60.9, 128.4, 128.5, 137.1, 140.8; ESI-HRMS (M + H)$^+$ m/z calcd for C$_{20}$H$_{35}$N$_2$ 303.2800, found 303.2797.

Preparation of 2-(4-(4-Octylphenethyl)piperazin-1-yl)ethanol (RB-008)

Compound RB-008 was prepared from 4-octylphenethyl methanesulfonate according to a coupling procedure similar to that described for compound RB-001, using 1-(2-hydroxyethyl)piperazine. Yield = 63%; $^1$H NMR (400 MHz, CDCl$_3$) δ 0.87 (t, $J = 6.9$ Hz, 3H), 1.26–1.30 (m, 10H), 1.55–1.62 (m, 2H), 2.49–2.62 (m, 14H), 2.76–2.80 (m, 2H), 3.63 (t, $J = 5.4$ Hz, 2H), 7.08–7.13 (m, 4H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 14.1, 22.7, 29.3, 29.4, 29.5, 31.6, 31.9, 33.2, 35.6, 52.8, 53.2, 57.7, 59.2, 60.6, 128.5, 128.6, 137.2, 140.8; ESI-HRMS (M + H)$^+$ m/z calcd for C$_{22}$H$_{39}$N$_2$O 347.3062, found 347.3061.

Preparation of 3-(4-(4-Octylphenethyl)piperazin-1-yl)propan-1-ol (RB-009)

Compound RB-009 was prepared from 4-octylphenethyl methanesulfonate according to a coupling procedure similar to that described for compound RB-001, using 1-(3-hydroxypropyl)piperazine. Yield = 75%; $^1$H NMR (400 MHz, CDCl$_3$) δ (t, $J = 6.8$ Hz, 3H), 1.25–1.32 (m, 10H), 1.54–1.62 (m, 2H), 1.75 (q, $J = 5.5$ Hz, 2H), 2.56 (t, $J = 7.8$ Hz, 3H), 1.25–1.32 (m, 10H), 1.54–1.62 (m, 2H), 1.75 (q, $J = 5.5$ Hz, 2H), 2.56 (t, $J = 7.8$ Hz, 3H).
Hz, 4H), 2.59–2.64 (m, 4H), 2.69 (t, J = 5.7 Hz, 4H), 2.75–2.79 (m, 4H), 3.81 (t, J = 5.2 Hz, 2H), 7.09 (s, 4H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 14.1, 22.7, 26.9, 29.3, 29.4, 29.5, 29.7, 31.6, 31.9, 33.0, 35.6, 52.8, 53.1, 58.6, 60.3, 128.4, 128.5, 137.0, 140.8; ESI-HRMS (M + H)$^+$ m/z calcd for C$_{23}$H$_{41}$N$_2$O 361.3219, found 361.3217.

**Preparation of 1’-(4-Octylphenethyl)-1,4’-bipiperidine (RB-010)**

![Chemical structure of RB-010](image)

Compound RB-010 was prepared from 4-octylphenethyl methanesulfonate according to a coupling procedure similar to that described for compound RB-001, using 1,4’-bipiperidine. Yield = 70%; $^1$H NMR (400 MHz, CDCl$_3$) δ 0.87 (t, J = 6.8 Hz, 3H), 1.26–1.29 (m, 10H), 1.42–1.45 (m, 2H), 1.54–1.70 (m, 8H), 1.81 (d, J = 12.3 Hz, 2H), 1.99 (dt, J = 11.7, 1.6 Hz, 2H), 2.30–2.36 (m, 1H), 2.53–2.58 (m, 8H), 2.74–2.79 (m, 2H), 3.07 (d, J = 11.6 Hz, 2H), 7.07–7.12 (m, 4H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 14.1, 22.7, 24.8, 26.3, 27.6, 29.3, 29.4, 29.5, 31.6, 31.9, 33.5, 35.6, 50.1, 53.6, 60.8, 62.9, 128.4, 128.6, 137.5, 140.6; ESI-HRMS (M + H)$^+$ m/z calcd for C$_{26}$H$_{45}$N$_2$ 385.3582, found 385.3577.

**Preparation of 1-Methyl-1-(4-octylphenethyl)pyrrolidinium Methanesulfonate (RB-011)**

![Chemical structure of RB-011](image)

To a solution of 4-octylphenethyl methanesulfonate (10 mg, 0.032 mmol) in 3 mL of acetonitrile was added 1-methylpyrrolidine (34.1 µL, 0.32 mmol). The reaction mixture was stirred at 50 °C for 12 h and concentrated. The residue was washed with hexane to give 12 mg (92%) of RB-011 as a yellow liquid; $^1$H NMR (400 MHz, CDCl$_3$) δ 0.88 (t, J = 6.8 Hz, 3H), 1.26–1.30 (m, 10H), 1.54–1.58 (m, 2H), 2.22 (m, 4H), 2.55 (t, J = 7.8 Hz, 2H), 2.75 (s, 3H), 3.01 (t, J = 8.2 Hz, 2H), 3.25 (s, 3H), 3.70–3.77 (m, 6H),
7.13 (d, J = 8.0 Hz, 2H), 7.22 (d, J = 8.0 Hz, 2H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 14.1, 21.5, 22.7, 29.3 (2C), 29.5, 30.1, 31.5, 31.9, 35.5, 48.2, 64.4, 64.6, 128.9, 129.1, 132.2, 142.3; ESI-HRMS (M)$^+$ m/z calcd for C$_{21}$H$_{37}$N$^+$ 303.2926, found 303.2875.

5 Preparation of 1-Methyl-1-(4-octylphenethyl)piperidinium Methanesulfonate (RB-012)

![Scheme 5](image)

To a solution of 4-octylphenethyl methanesulfonate (10 mg, 0.032 mmol) in 3 mL of acetonitrile was added 1-methylpiperidine (38.9 µL, 0.32 mmol). The reaction mixture was stirred at 50 °C for 12 h and concentrated. The residue was washed with hexane to give 12 mg (90%) of RB-012 as a yellow liquid; $^1$H NMR (400 MHz, CDCl$_3$) δ 0.88 (t, J = 6.8 Hz, 3H), 1.26–1.29 (m, 10H), 1.57 (t, J = 7.3 Hz, 2H), 1.72–1.88 (m, 6H), 2.55 (t, J = 7.7 Hz, 2H), 2.75 (s, 3H), 3.00–3.05 (m, 2H), 3.30 (s, 3H), 3.52–3.56 (m, 2H), 3.64–3.70 (m, 4H), 7.15 (d, J = 8.0 Hz, 2H), 7.12 (d, J = 8.0 Hz, 2H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 14.1, 20.2, 20.7, 22.7, 28.2, 29.3 (2C), 29.5, 31.5, 31.9, 35.5, 39.7, 48.5, 61.0, 128.9, 129.1, 132.1, 142.3; ESI-HRMS (M)$^+$ m/z calcd for C$_{22}$H$_{39}$N$^+$ 317.3082, found 317.3032.

Preparation of 1-Methyl-1-(4-octylphenethyl)azepanium Iodide (RB-013)

To a solution of RB-003 (10 mg, 0.032 mmol) in MeCN (3 mL) was added K₂CO₃ (22 mg, 0.16 mmol) at rt. After the suspension was stirred for 10 min, MeI (10 μL, 0.16 mmol) was added. The reaction mixture was stirred overnight at rt. The reaction mixture was diluted with water, and the product was extracted with EtOAc. The extract was washed with brine, dried, and evaporated. The residue was washed with hexane to give 12 mg (84%) of RB-013 as a colorless oil; ^1^H NMR (400 MHz, CDCl₃) δ 0.88 (t, J = 6.8 Hz, 3H), 1.24–1.32 (m, 10H), 1.54–1.57 (m, 2H), 1.72–1.77 (m, 4H), 1.91–1.96 (m, 4H), 2.54 (t, J = 7.7 Hz, 2H), 3.11–3.15 (m, 2H), 3.42 (s, 3H), 3.71–3.73 (m, 4H), 3.77–3.81 (m, 2H), 7.12 (d, J = 8.0 Hz, 2H), 7.30 (d, J = 8.0 Hz, 2H); ^1^C NMR (100 MHz, CDCl₃) δ 14.2, 22.1, 22.7, 27.3, 29.0, 29.3, 29.5, 29.7, 31.5, 31.9, 35.6, 51.5, 65.1, 65.6, 129.1, 129.2, 131.9, 142.3; ESI-HRMS (M⁺) m/z calcd for C₂₃H₄₀N⁺ 330.3161, found 331.3153.

Preparation of 1,4-Dimethyl-1-(4-octylphenethyl)piperidinium Iodide (RB-014)

Compound RB-014 was prepared from RB-004 according to a coupling procedure similar to that described for compound RB-013. Yield = 90%; ^1^H NMR (400 MHz, CDCl₃) δ 0.88 (t, J = 6.8 Hz, 3H), 1.02 (d, J = 6.5 Hz, 3H), 1.22–1.35 (m, 10H), 1.51–1.64 (m, 5H), 1.80–1.84 (m, 2H), 2.53 (t, J = 7.8 Hz, 2H), 3.08–3.12 (m, 2H), 3.26 (s, 3H), 3.60–3.82 (m, 4H), 4.04–4.10 (m, 2H), 7.11 (d, J = 8.0 Hz, 2H), 7.33 (d, J = 8.0 Hz, 2H); ^1^C NMR (100 MHz, CDCl₃) δ 14.2, 20.9, 22.7, 28.1, 29.3, 29.4, 29.7, 31.5, 31.9, 35.6, 44.9, 61.0, 67.9, 129.1, 129.4, 131.4, 142.2; ESI-HRMS (M⁺) m/z calcd for C₂₃H₄₀N⁺ 330.3161, found 330.3156.
Preparation of 4-Hydroxy-1-methyl-1-(4-octylphenethyl)piperidinium Iodide (RB-015)

Compound RB-015 was prepared from RB-005 according to a coupling procedure similar to that described for compound RB-013. Yield = 84%; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 0.87 (t, $J = 6.7$ Hz, 3H), 1.25–1.28 (m, 10H), 1.50–1.57 (m, 2H), 2.02–2.11 (m, 3H), 2.23–2.28 (m, 2H), 2.51 (t, $J = 6.9$ Hz, 2H), 3.04–3.11 (m, 2H), 3.37 (d, $J = 9.2$ Hz, 3H), 3.62–3.76 (m, 6H), 4.18–4.24 (m, 1H), 7.13 (dd, $J = 2.1$, 7.2 Hz, 2H), 7.29 (dd, $J = 2.2$, 6.5 Hz, 2H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 14.1, 22.7, 27.8, 28.2, 28.4, 28.7, 29.3, 29.4, 29.5, 31.6, 31.9, 35.6, 57.6, 58.4, 129.1, 129.2, 131.9, 142.3; ESI-HRMS (M$^+$) m/z calced for C$_{22}$H$_{38}$NO$^+$ 332.2953, found 332.2946.

Preparation of N,N-Dimethyl-2-(4-octylphenyl)-N-((tetrahydrofuran-2-yl)methyl)ethanaminium Iodide (RB-016)

Compound RB-016 was prepared from RB-006 according to a coupling procedure similar to that described for compound RB-013. Yield = 88%; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 0.87 (t, $J = 6.7$ Hz, 3H), 1.26–1.29 (m, 10H), 1.55–1.67 (m, 6H), 1.89–1.99 (m, 2H), 2.25–2.54 (m, 2H), 2.56 (t, $J = 7.7$ Hz, 2H), 3.03–3.13 (m, 2H), 3.47 (s, 3H), 3.48 (s, 3H), 3.81–3.88 (m, 1H), 3.93–3.99 (m, 1H), 4.34–4.39 (m, 1H), 7.13 (d, $J = 8.0$ Hz, 2H), 7.22 (d, $J = 8.0$ Hz, 2H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 14.1, 22.7, 24.9, 29.2, 29.3, 29.5, 30.4, 31.5, 31.9, 35.6, 52.4, 60.2, 69.3, 72.9, 129.0, 129.2, 131.6, 142.4; ESI-HRMS (M$^+$) m/z calced for C$_{23}$H$_{40}$NO$^+$ 346.3110, found 346.3102.
Preparation of \( N,N\)-Dimethyl-\(N\)-(4-octylphenethyl)cyclohexanaminium Mesylate (RB-017)

\[
\begin{align*}
\text{Compound } \text{RB-017} &\text{ was prepared from 4-octylphenethyl methanesulfonate according to a coupling procedure similar to that described for compound RB-011, using } \text{N,}\text{N-dimethylcyclohexylamine. Yield } = 76\%; \quad ^1\text{H NMR (400 MHz, CDCl}_3\text{) } \delta 0.88 (t, J = 6.7 \text{ Hz}, 3H), 1.26–1.29 (m, 10H), 1.35–1.45 (m, 5H), 1.51–1.58 (m, 2H), 1.96 (d, J = 12.1 \text{ Hz}, 2H), 2.20 (d, J = 11.6 \text{ Hz}, 2H), 7.55 (t, J = 7.7 \text{ Hz}, 2H), 2.73 (s, 3H), 3.04–3.08 (m, 2H), 3.25 (s, 6H), 3.48 (m, 1H), 3.58–3.62 (m, 2H), 7.12 (d, J = 7.9, 2H), 7.22 (d, J = 7.9, 2H); \quad ^{13}\text{C NMR (100 MHz, CDCl}_3\text{) } \delta 14.1, 22.7, 24.7, 24.8, 24.9, 25.3, 26.2, 26.6, 28.6, 29.3, 29.5, 31.5, 31.9, 35.3, 39.7, 48.5, 63.1, 72.2, 128.9, 129.1, 132.4, 142.2; \quad \text{ESI-HRMS (M}^+\text{) } m/z \text{ calcd for C}_{24}\text{H}_{42}\text{N}^+ 344.3317, \text{ found 344.3313.}
\end{align*}
\]

Preparation of \( N,N\)-Dimethyl-\(N\)-(4-octylphenethyl)butan-1-aminium Mesylate (RB-018)

\[
\begin{align*}
\text{Compound } \text{RB-018} &\text{ was prepared from 4-octylphenethyl methanesulfonate according to a coupling procedure similar to that described for compound RB-011, using } \text{N-n-butylidemethylamine. Yield } = 65\%; \quad ^1\text{H NMR (400 MHz, CDCl}_3\text{) } \delta 0.88 (t, J = 6.7 \text{ Hz}, 3H), 0.97 (t, J = 7.3 \text{ Hz}, 3H), 1.21–1.29 (m, 10H), 1.27–1.40 (m, 2H), 1.54–1.58 (m, 2H), 1.59–1.68 (m, 2H), 2.54 (t, J = 7.7 \text{ Hz}, 2H), 2.72 (s, 3H), 3.01–3.05 (m, 2H), 3.30 (m, 3H), 3.45–3.49 (m, 2H), 3.58–3.62 (m, 2H), 7.12 (d, J = 7.9Hz, 2H), 7.21 (d, J = 8.0 \text{ Hz}, 2H); \quad ^{13}\text{C NMR (100 MHz, CDCl}_3\text{) } \delta 14.1, 19.6, 22.6, 24.6, 28.8, 29.2, 29.3, 29.4, 31.5, 31.9, 35.5, 39.7, 51.0, 63.7, 64.2, 128.9, 129.1, 132.2, 142.2; \quad \text{ESI-HRMS (M}^+\text{) } m/z \text{ calcd for C}_{22}\text{H}_{40}\text{N}^+ 318.3161, \text{ found 318.3156.}
\end{align*}
\]
Preparation of 1-(4-Octylphenethyl)piperidin-3-ol (RB-019)

Compound RB-019 was prepared from 4-octylphenethyl methanesulfonate according to a coupling procedure similar to that described for compound RB-005, using 3-hydroxypiperidine. Yield = 82%; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 0.88 (t, $J = 6.9$ Hz, 3H), 1.20–1.30 (m, 10H), 1.57–1.60 (m, 5H), 1.86–1.89 (m, 1H), 2.39–2.43 (m, 1H), 2.54–2.67 (m, 7H), 2.78–2.81 (m, 2H), 7.10 (s, 4H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 14.1, 21.4, 22.7, 29.3, 29.4, 29.5, 31.6, 31.9, 32.7, 35.6, 53.6, 60.1, 60.3, 66.0, 128.5, 128.6, 136.9, 140.8; ESI-HRMS (M + H)$^+$ $m/z$ calcd for C$_{21}$H$_{36}$NO 318.2797, found 318.2792.

Preparation of (1-(4-Octylphenethyl)piperidin-4-yl)methanol (RB-020)

Compound RB-020 was prepared from 4-octylphenethyl methanesulfonate according to a coupling procedure similar to that described for compound RB-005, using 4-piperidine methanol. Yield = 87%; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 0.88 (t, $J = 6.8$ Hz, 3H), 1.21–1.32 (m, 10H), 1.54–1.61 (m, 2H), 1.69–1.75 (m, 3H), 1.89–1.92 (m, 2H), 2.39–2.48 (m, 2H), 2.56 (t, $J = 7.8$ Hz, 2H), 2.88–2.92 (m, 2H), 3.01–3.05 (m, 2H), 3.36 (d, $J = 11.0$ Hz, 2H), 3.56 (d, $J = 5.4$ Hz, 2H), 7.10–7.14 (m, 4H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 14.1, 22.7, 27.0, 29.3, 29.4, 29.5, 31.3, 31.5, 31.9, 35.6, 37.4, 53.1, 59.7, 66.7, 128.6, 128.8, 135.0, 141.5; ESI-HRMS (M + H)$^+$ $m/z$ calcd for C$_{22}$H$_{38}$NO 332.2953, found 332.2948.

(Hex-5-ynylsulfonyl)benzene. To a solution of 6-chloro-1-hexyne (100 mg, 0.86 mmol) in 12 mL of THF/DMF (2:1) was added benzenesulfinic acid sodium salt (422 mg, 2.6 mmol) in a sealed tube. The reaction mixture was stirred at 80 °C for 3 d. The reaction mixture was then diluted with water, and the product was extracted with EtOAc. The extract was washed with brine, dried, and evaporated. Purification by silica gel chromatography, elution with hexane/EtOAc (3:1), afforded 17 mg (56%) of hex-5-ynylsulfonyl)benzene as a colorless oil; ¹H NMR (400 MHz, CDCl₃) δ 1.61 (quin, J = 7.3 Hz, 2H), 1.81–1.89 (m, 2H), 1.95 (t, J = 2.6 Hz, 1H), 2.19 (dt, J = 6.9, 2.6 Hz, 2H), 3.11–3.15 (m, 2H), 7.56–7.60 (m, 2H), 7.65–7.69 (m, 1H), 7.90–7.92 (m, 2H); ¹³C NMR (100 MHz, CDCl₃) δ 17.9, 21.8, 26.8, 55.6, 69.3, 83.0, 128.0, 129.3, 133.7, 139.0, 129.3, 133.7, 139.0; ESI-HRMS (M + Na)⁺ m/z calcd for C₁₂H₁₄NaO₂S 245.0612, found 245.0612.

2-(4-(6-(Phenylsulfonyl)hex-1-ynyl)phenyl)ethanol. To a deaerated solution of 2-(4-bromophenyl)ethanol (100 mg, 0.50 mmol), bis(triphenylphosphine)palladium dichloride (29 mg, 0.025 mmol), and copper(I) iodide (4.8 mg, 0.025 mmol) in anhydrous triethylamine (TEA, 10 mL) was added (hex-5-ynylsulfonyl)benzene (221 mg, 0.99 mmol) at rt. The reaction mixture was heated at 50 °C for 8 h. After saturated aqueous ammonium chloride solution was added, the mixture was extracted with EtOAc. The combined solution was washed with water, brine, and dried. Flash column
chromatography with hexanes/EtOAc (1:2) as the eluent gave the product (114 mg, 67%) as a yellow oil; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 1.63 (br s, 1H), 1.68 (quin, $J = 7.3$ Hz, 2H), 1.87–1.95 (m, 2H), 2.41 (t, $J = 6.9$ Hz, 2H), 2.85 (t, $J = 6.6$ Hz, 2H), 3.14–3.18 (m, 2H), 3.84 (dd, $J = 6.1$, 10.9 Hz, 2H), 7.14 (d, $J = 8.2$ Hz, 2H), 7.26 (d, $J = 8.1$ Hz, 2H), 7.53–7.57 (m, 2H), 7.63–7.67 (m, 1H), 7.91–7.93 (m, 2H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 19.0, 22.0, 27.1, 39.0, 55.9, 63.5, 81.4, 88.4, 121.7, 128.1, 129.0, 129.3, 131.7, 133.8, 138.4, 139.1; ESI-HRMS (M + Na)$^+$ m/z calcld for C$_{20}$H$_{22}$NaO$_3$S 365.1187, found 365.1183.

10 Preparation of 2-(4-(6-(Phenylsulfonyl)hexyl)phenyl)ethanol

2-(4-(6-(Phenylsulfonyl)hex-1-ynyl)phenyl)ethanol (40 mg, 0.12 mmol) was dissolved in EtOAc (5 mL), and 10% Pd/C (40 mg, 100 wt %) was added. The reaction mixture was hydrogenated at rt for 12 h. The catalyst was removed by filtration through a pad of Celite and rinsed with EtOAc. The product was obtained without purification as a colorless oil; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 1.22–1.41 (m, 4H), 1.56 (quin, $J = 7.6$ Hz, 2H), 1.65 (br s, 1H), 1.66–1.74 (m, 2H), 2.54 (t, $J = 7.6$ Hz, 2H), 2.83 (t, $J = 6.6$ Hz, 2H), 3.05–3.09 (m, 2H), 3.84 (t, $J = 6.6$ Hz, 2H), 7.08 (d, $J = 8.0$ Hz, 2H), 7.13 (d, $J = 8.0$ Hz, 2H), 7.55–7.59 (m, 2H), 7.64–7.68 (m, 1H), 7.89–7.91 (m, 2H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 22.6, 28.1, 28.6, 31.0, 35.3, 38.8, 56.2, 63.7, 128.0, 128.6, 129.0, 129.6, 133.7, 135.8, 139.1, 140.5; ESI-HRMS (M + Na)$^+$ m/z calcld for C$_{20}$H$_{26}$NaO$_3$S 369.1500, found 369.1499.

Preparation of 4-(6-(Phenylsulfonyl)hexyl)phenethyl Methanesulfonate

The mesylate was prepared from the alcohol in 92% yield; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 1.24–1.42 (m, 4H), 1.55 (quin, $J = 7.6$ Hz, 2H), 1.67–1.74 (m, 2H), 2.55 (t, $J = 7.6$ Hz, 2H), 2.85 (s, 3H), 3.02 (t, $J = 7.0$ Hz, 2H), 3.05–3.09 (m, 2H), 4.40 (t, $J = 7.0$ Hz, 2H), 7.09 (d, $J = 8.2$ Hz, 2H), 7.13 (d, $J = 8.1$ Hz, 2H), 7.53–7.59 (m, 2H), 7.64–7.68 (m, 1H), 7.89–7.91 (m, 2H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 22.6, 28.1, 28.6, 31.0, 35.3, 37.3,
Preparation of 1-(4-(6-(Phenylsulfonyl)hexyl)phenethyl)piperidin-4-ol (RB-021)

**RB-021** was prepared according to a coupling procedure similar to that described for RB-005. Yield = 78%; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 1.26–1.32 (m, 4H), 1.38 (quin, $J$ = 7.4 Hz, 2H), 1.55 (quin, $J$ = 7.5 Hz, 2H), 1.66–1.78 (m, 5H), 2.08–2.18 (m, 2H), 2.53 (t, $J$ = 7.6 Hz, 2H), 2.78–2.83 (m, 2H), 2.92–2.94 (m, 2H), 2.98–3.08 (m, 4H), 3.96–3.98 (m, 2H), 1.55 (quin, $J$ = 7.4 Hz, 2H), 7.11 (d, $J$ = 8.0 Hz, 2H), 7.12 (d, $J$ = 8.0 Hz, 2H), 7.55–7.59 (m, 2H), 7.64–7.68 (m, 2H), 7.89–7.91 (m, 2H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 22.6, 28.1, 28.5, 31.0, 33.2, 34.4, 35.3, 50.4, 51.1, 56.2, 60.1, 67.7, 128.1, 128.6, 129.3, 133.7, 139.1, 140.6; ESI-HRMS (M + H)$^+$ m/z calcd for C$_{25}$H$_{36}$NO$_3$S 430.2416, found 430.2413.

Preparation of 1-(4-(6-(Phenylsulfonyl)hexyl)phenethyl)piperidine (RB-022)

**RB-022** was prepared according to a coupling procedure similar to that described for compound RB-002. Yield = 83%; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 1.25–1.42 (m, 6H), 1.51–1.59 (m, 4H), 1.66–1.74 (m, 4H), 1.89–1.93 (m, 4H), 2.53 (t, $J$ = 7.6 Hz, 2H), 2.95–3.08 (m, 6H), 7.07 (d, $J$ = 8.0 Hz, 2H), 7.12 (d, $J$ = 8.0 Hz, 2H), 7.56–7.59 (m, 2H), 7.64–7.68 (m, 1H), 7.89–7.91 (m, 2H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 22.6, 23.0, 24.0, 28.2, 28.6, 29.7, 31.0, 35.3, 39.5, 53.9, 54.4, 56.3, 59.4, 128.1, 128.4, 129.3, 133.7, 193.2; ESI-HRMS (M + H)$^+$ m/z calcd for C$_{25}$H$_{36}$NO$_3$S 414.2466, found 414.2468.
Preparation of 1-(4-Octylbenzyl)piperidin-4-ol (RB-023)

4-(Octylphenyl)methanol was prepared in two steps from 4-iodobenzyl alcohol; first, a Sonogashira reaction with 1-octyne afforded (4-(oct-1-ynyl)phenylmethanol as a yellow oil, and then catalytic hydrogenation of the triple bond provided 4-(octylphenyl)methanol as a colorless oil. To a solution of 4-(octylphenyl)methanol (37 mg, 0.17 mmol) and triethylamine (0.23 mL, 1.7 mmol) in CH$_2$Cl$_2$ (5 mL) at 0 °C was added methanesulfonyl chloride (40 µL, 0.50 mmol). After being stirred at rt for 3 h, the reaction mixture was evaporated, diluted with water, and the product was extracted with EtOAc. The extract was washed with brine, dried, and evaporated. To a solution of the reaction mixture (0.17 mmol) in MeCN (3 mL) was added 4-hydroxypiperidine (86 mg, 0.85 mmol). The reaction mixture was stirred at 50 °C for 12 h and concentrated. Purification by silica gel chromatography, eluting with CH$_2$Cl$_2$/MeOH (5:1), gave 35 mg (69%, 2 steps) of RB-023 as a colorless oil; $^1$H NMR (400 MHz, CDCl$_3$) δ 0.88 (t, $J$ = 6.8 Hz, 3H), 1.23–1.30 (m, 10H), 1.56–1.73 (m, 4H), 1.97–2.06 (m, 2H), 2.35–2.47 (m, 2H), 2.59 (t, $J$ = 7.7 Hz, 2H), 2.85–2.90 (m, 2H), 3.64 (s, 2H), 3.78–3.81 (m, 1H), 7.15 (d, $J$ = 7.9 Hz, 2H), 7.27 (d, $J$ = 8.0 Hz, 2H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 14.1, 22.7, 29.3, 29.4, 29.5, 31.5, 31.9, 33.2, 35.7, 50.1, 62.2, 128.5, 129.7, 137.2, 142.8; ESI-HRMS (M + H)$^+$ m/z calcd for C$_{20}$H$_{34}$NO 304.2640, found 304.2637.
Preparation of 1-(3-(4-Octylphenyl)propyl)piperidin-4-ol (RB-024)

This product was prepared from 1-bromo-4-n-octylbenzene in three steps. First, 3-(4-octylphenyl)prop-2-yn-1-ol was prepared from 1-bromo-4-n-octylbenzene and propargyl alcohol by a Sonogashira reaction procedure; yield = 82%; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 0.86 (t, $J = 6.8$ Hz, 3H), 1.22–1.30 (m, 10H), 1.57–1.60 (m, 2H), 2.59 (t, $J = 7.7$ Hz, 2H), 4.49 (s, 2H), 7.11 (d, $J = 7.8$ Hz, 2H), 7.34 (d, $J = 7.7$ Hz, 2H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 14.1, 22.7, 29.2, 29.4, 31.9, 35.9, 51.7, 85.9, 86.5, 119.6, 128.4, 131.6, 143.7; ESI-HRMS (M + H)$^+$ $m/z$ calcd for C$_{17}$H$_{25}$O 245.1905, found 245.1903. Then, catalytic hydrogenation provided 3-(4-octylphenyl)propan-1-ol; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 0.88 (t, $J = 6.8$ Hz, 3H), 1.24–1.30 (m, 10H), 1.59 (quin, $J = 7.4$ Hz, 2H), 1.88 (quin, $J = 6.5$ Hz, 2H), 2.56 (t, $J = 7.7$ Hz, 2H), 2.67 (t, $J = 7.7$ Hz, 2H), 3.66 (t, $J = 6.4$ Hz, 2H), 7.10 (s, 4H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 14.1, 22.7, 29.3, 29.4, 29.5, 31.6, 31.7, 31.9, 34.3, 35.6, 62.4, 128.3, 128.4, 138.9, 140.5; ESI-HRMS (M + H)$^+$ $m/z$ calcd for C$_{17}$H$_{25}$O 249.2218, found 249.2210. Finally, RB-024 was prepared from 3-(4-octylphenyl)propan-1-ol according to a procedure similar to that described for RB-023; yield = 73%; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 0.87 (t, $J = 6.8$ Hz, 3H), 1.25–1.30 (m, 10H), 1.55–1.65 (m, 4H), 1.79–1.92 (m, 4H), 2.16–2.20 (m, 2H), 2.40–2.43 (m, 2H), 2.53–2.63 (m, 4H), 2.80–2.83 (m, 2H), 3.67–3.69 (m, 1H), 7.08 (s, 4H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 14.1, 22.7, 28.5, 29.3, 29.4, 29.5, 31.6, 31.9, 33.3, 34.0, 35.6, 50.9, 57.9, 128.2, 128.4, 138.9, 140.4; ESI-HRMS (M + H)$^+$ $m/z$ calcd for C$_{22}$H$_{38}$NO 332.2953, found 332.2951.
Preparation of 1-(4-(4-Octylphenyl)butyl)piperidin-4-ol (RB-025)

![Chemical Structure](image)

Compound **RB-025** was prepared from 4-(4-octylphenyl)butan-1-ol according to a procedure similar to that described for compound **RB-023**: yield = 67% (2 steps); $^1$H NMR (400 MHz, CDCl$_3$) δ 0.88 (t, $J$ = 6.9 Hz, 3H), 1.24–1.33 (m, 10H), 1.55–1.68 (m, 4H), 1.80–1.88 (m, 4H), 2.21–2.26 (m, 2H), 2.56 (t, $J$ = 7.8 Hz, 2H), 2.61 (t, $J$ = 7.5 Hz, 2H), 2.71–2.89 (m, 4H), 3.11 (t, $J$ = 9.0 Hz, 2H), 3.97–4.01 (m, 1H), 7.05 (d, $J$ = 8.4 Hz, 2H), 7.09 (d, $J$ = 8.4 Hz, 2H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 14.1, 22.7, 28.9, 29.3, 29.4, 29.5, 31.6, 31.9, 34.9, 35.6, 57.5, 128.2, 128.5, 138.6, 140.7; ESI-HRMS (M + H)$^+$ m/z calcd for C$_{23}$H$_{40}$NO 346.3110, found 346.3107.

**Preparation of RB-026 – RB-033**

Products RB-026, RB-027, and RB-028 were prepared in three steps from 2-(4-bromophenyl)ethanol. First, 2-(4-(hex-1-ynyl)phenyl)ethanol was prepared from 2-(4-bromophenyl)ethanol and 1-hexyne by a Sonogashira procedure; yield = 60%; $^1$H NMR (400 MHz, CDCl$_3$) δ 0.95 (t, $J$ = 7.3 Hz, 3H), 1.43–1.52 (m, 2H), 1.55–1.62 (m, 2H), 2.40 (t, $J$ = 7.0 Hz, 2H), 2.85 (t, $J$ = 6.5 Hz, 2H), 3.84 (t, $J$ = 6.5 Hz, 2H), 7.14 (d, $J$ = 8.1 Hz, 2H), 7.34 (t, $J$ = 8.1 Hz, 2H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 13.7, 19.1, 22.0, 29.7, 30.9, 39.0, 63.5, 80.3, 90.2, 122.3, 128.9, 131.7, 137.9; ESI-HRMS (M + H)$^+$ m/z calcd for C$_{18}$H$_{19}$NO 203.1436, found 203.1433. Similarly, 2-(4-(dodec-1-ynyl)phenyl)ethanol was prepared from 2-(4-bromophenyl)ethanol and 1-decyne by a Sonogashira reaction; yield = 62%; $^1$H NMR (400 MHz, CDCl$_3$) δ 0.88 (t, $J$ = 6.8 Hz, 3H), 1.23–1.30 (m, 12H), 1.40–1.45 (m, 2H), 1.59 (quin, $J$ = 7.3 Hz, 2H), 2.38 (t, $J$ = 7.1 Hz, 2H), 2.81 (t, $J$ = 6.6 Hz, 2H), 3.79 (t, $J$ = 6.5 Hz, 2H), 7.11 (d, $J$ = 8.2 Hz, 2H), 7.32 (d, $J$ = 8.1 Hz, 2H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 14.1, 19.4, 22.7, 24.9, 28.8, 29.0, 29.2, 29.4, 29.6, 31.9, 39.0, 63.4, 80.4, 90.2, 122.3, 128.6, 131.4, 138.0; ESI-HRMS (M + H)$^+$ m/z calcd for C$_{20}$H$_{31}$O

66
287.2375, found 287.2371. Catalytic hydrogenation of these alkynes afforded the corresponding saturated alcohols, 2-(4-hexylphenyl)ethanol and 2-(4-dodecylphenyl)ethanol. Data for 2-(4-hexylphenyl)ethanol: $^1$H NMR (400 MHz, CDCl$_3$) δ 0.88 (t, $J = 6.6$ Hz, 3H), 1.22–1.37 (m, 6H), 1.55–1.63 (m, 2H), 2.57 (t, $J = 7.8$ Hz, 2H), 2.84 (t, $J = 6.6$ Hz, 2H), 3.84 (t, $J = 6.5$ Hz, 2H), 7.13 (s, 4H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 14.1, 22.6, 29.0, 31.5, 31.7, 35.6, 38.8, 63.8, 128.6, 128.9, 135.5, 141.2; ESI-HRMS (M + H)$^+$ m/z calcd for C$_{14}$H$_{23}$O 207.1749, found 207.1725. Data for 2-(4-dodecylphenyl)ethanol: $^1$H NMR (400 MHz, CDCl$_3$) δ 0.88 (t, $J = 6.8$ Hz, 3H), 1.23–1.31 (m, 18H), 1.57–1.60 (m, 2H), 2.56 (t, $J = 7.8$ Hz, 2H), 2.81 (t, $J = 6.6$ Hz, 2H), 3.81 (t, $J = 6.6$ Hz, 2H), 7.12 (s, 4H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 14.1, 22.7, 29.4, 29.6, 29.7, 31.6, 31.9, 35.6, 38.8, 63.7, 128.6, 128.9, 135.5, 141.2; $^{13}$C NMR (100 MHz, CDCl$_3$) δ 21.0, 32.3, 33.1, 39.5, 50.5, 59.9, 66.0, 128.6, 129.3, 135.9, 136.0; ESI-HRMS (M + Na)$^+$ m/z calcd for C$_{29}$H$_{34}$ONa 313.2507, found 313.2502.

**Preparation of 1-(4-Methylphenethyl)piperidin-4-ol (RB-026)**

![RB-026](image)

Compound **RB-026** was prepared from 2-(4-methylphenyl)ethanol according to a procedure similar to that described for **RB-023**; yield = 69%; $^1$H NMR (400 MHz, CDCl$_3$) δ 1.69–1.77 (m, 2H), 1.99–2.04 (m, 2H), 2.31 (s, 3H), 2.48–2.52 (2H), 2.72–2.76 (m, 2H), 2.79 (s, 1H), 2.84–2.88 (m, 2H), 2.98–3.03 (m, 2H), 3.78–3.84 (m, 1H), 7.10 (s, 4H); ESI-HRMS (M + H)$^+$ m/z calcd for C$_{14}$H$_{22}$NO 220.1701, found 220.1699.
Preparation of 1-(4-Hexylphenethyl)piperidin-4-ol (RB-027)

Compound RB-027 was prepared from 2-(4-hexaphenyl)ethanol according to a procedure similar to that described for RB-023; yield = 79%; $^1$H NMR (400 MHz, CDCl$_3$) δ 0.88 (t, $J = 6.5$ Hz, 3H), 1.26–1.36 (m, 6H), 1.58 (quin, $J = 7.4$ Hz, 2H), 1.75–1.80 (m, 2H), 2.08–2.13 (m, 2H), 2.56 (t, $J = 7.7$ Hz, 2H), 2.74–2.77 (m, 2H), 2.90–2.94 (m, 2H), 3.00–3.04 (m, 2H), 3.86–3.90 (m, 1H), 7.11 (s, 4H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 14.1, 22.6, 29.0, 31.5, 31.7, 35.6, 50.1, 59.9, 128.5, 128.6, 141.2; ESI-HRMS (M + H)$^+$ $m/z$ calcd for C$_{19}$H$_{32}$NO 290.2484, found 290.2478.

Preparation of 1-(4-Dodecylphenethyl)piperidin-4-ol (RB-028)

Compound RB-028 was prepared from 2-(4-dodecylphenyl)ethanol according to a procedure similar to that described for RB-023; yield = 75%; $^1$H NMR (400 MHz, CDCl$_3$) δ 0.88 (t, $J = 6.6$ Hz, 3H), 1.23–1.33 (m, 18H), 1.56–1.60 (m, 2H), 1.67–1.72 (m, 2H), 1.98–2.01 (m, 2H), 2.34–2.39 (m, 2H), 2.56 (t, $J = 7.4$ Hz, 2H), 2.64–2.68 (m, 2H), 2.81–2.84 (m, 2H), 2.91–2.95 (m, 2H), 3.75–3.79 (m, 1H), 7.10 (s, 4H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 14.1, 22.7, 29.4, 29.5, 29.6, 29.7, 31.6, 31.9, 32.8, 35.6, 50.6, 60.3, 128.5, 136.7, 140.9; ESI-HRMS (M + H)$^+$ $m/z$ calcd for C$_{25}$H$_{44}$NO 374.3423, found 374.3414.
Preparation of 4-Azido-1-(4-methylphenethyl)piperidine (RB-029)

To a solution of RB-026 (115 mg, 0.52 mmol) and triethylamine (0.73 mL, 5.24 mmol) in CH$_2$Cl$_2$ (5 mL) at 0 °C was added methanesulfonyl chloride (0.12 mL, 1.57 mmol). After being stirred at rt for 4 h, the reaction mixture was evaporated, diluted with water, and the product was extracted with EtOAc. The extract was washed with brine, evaporated, and dried. To a solution of reaction mixture in 5 mL of DMF was added sodium azide (170 mg, 2.62 mmol). The reaction mixture was stirred at 80 °C for 12 h and then concentrated. The residue was dissolved in EtOAc and the organic phase was evaporated and dried. Purification by silica gel chromatography, eluting with hexane/EtOAc (1/1), gave 79 mg (62%) of RB-029 as a colorless oil; $^{1}$H NMR (400 MHz, CDCl$_3$) δ 1.69–1.78 (m, 2H), 1.96–1.99 (m, 2H), 2.30–2.35 (m, 2H), 2.31 (s, 3H), 2.60–2.65 (m, 2H), 2.76–2.80 (m, 2H), 2.86–2.89 (m, 2H), 3.44–3.48 (m, 1H), 7.09 (s, 4H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 21.0, 29.4, 30.5, 33.0, 50.9, 57.3, 60.4, 128.6, 129.2, 132.4, 135.7, 136.7; ESI-HRMS (M + H)$^+$ m/z calcd for C$_{14}$H$_{21}$N$_4$ 245.1766, found 245.1763.

Preparation of 4-Azido-1-(4-octylphenethyl)piperidine (RB-030)

Compound RB-030 was prepared from RB-005 according to a procedure similar to that described for RB-029; yield = 71%; $^{1}$H NMR (400 MHz, CDCl$_3$) δ 0.87 (t, $J$ = 6.8 Hz, 3H), 1.24–1.31 (m, 10H), 1.58 (quin, $J$ = 7.2 Hz, 2H), 1.67–1.76 (m, 2H), 1.93–1.97


(m, 2H), 2.24–2.29 (m, 2H), 2.54–2.61 (m, 2H), 2.75–2.79 (m, 2H), 2.84–2.90 (m, 2H),
3.40–3.46 (m, 1H), 7.10 (s, 4H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 14.1, 22.7, 29.3, 29.4,
29.5, 29.7, 30.3, 30.7, 31.6, 31.9, 33.2, 35.6, 51.1, 57.6, 60.5, 128.5, 137.2, 140.8; ESI-
HRMS (M + H)$^+$ m/z calced for C$_{21}$H$_{35}$N$_4$ 343.2862, found 343.2857.

### Preparation of 1-(4-Methylphenethyl)piperidin-4-amine (RB-031)

![RB-031](image)

To a solution of RB-029 (30 mg, 0.12 mmol) in MeOH/CH$_2$Cl$_2$ (3/1, 3 mL) was
added 10% Pd/C (50 wt %). The reaction mixture was hydrogenated at rt for 12 h. The
catalyst was removed by filtration through a pad of Celite, which was rinsed with
MeOH/CH$_2$Cl$_2$ (3/1). The residue was washed with EtOAc/hexane (1/1), evaporated, and
dried. RB-031 was obtained as a white solid; $^1$H NMR (400 MHz, CD$_3$OD) $\delta$ 1.82–1.90
(m, 2H), 2.17 (d, $J = 10.2$ Hz, 2H), 2.28 (s, 3H), 2.34 (t, $J = 10.6$ Hz, 2H), 2.68–2.71 (m,
2H), 2.79–2.83 (m, 2H), 3.13 (d, $J = 11.2$ Hz, 2H), 3.13–3.20 (m, 1H), 7.07 (s, 4H); $^{13}$C
NMR (100 MHz, CDCl$_3$) $\delta$ 21.0, 30.7, 32.4, 48.2, 51.4, 59.8, 128.6, 129.2, 135.8, 136.0;
ESI-HRMS (M + H)$^+$ m/z calced for C$_{14}$H$_{23}$N$_2$ 219.1861, found 219.1858.

### Preparation of 1-(4-Octylphenethyl)piperidin-4-amine (RB-032)

![RB-032](image)

Compound RB-032 was prepared from RB-030 according to a procedure similar
to that described for RB-031; $^1$H NMR (400 MHz, CD$_3$OD) $\delta$ 0.91 (t, $J = 6.8$ Hz, 3H),
1.28–1.34 (m, 10H), 1.61 (quin, $J = 6.5$ Hz, 2H), 2.06–2.15 (m, 2H), 2.32 (d, $J = 13.2$ Hz,
2H), 2.60 (t, $J = 7.52$ Hz, 2H), 3.08–3.14 (m, 2H), 3.24 (t, $J = 11.5$ Hz, 2H), 3.35–3.38
Preparation of 1-(4-Dodecylphenethyl)piperidin-4-amine (RB-033)

To a solution of RB-028 (20 mg, 0.050 mmol) and triethylamine (70 µL, 0.54 mmol) in CH₂Cl₂ (3 mL) at 0 °C was added methanesulfonyl chloride (10 µL, 0.15 mmol). After being stirred at rt for 4 h, the reaction mixture was evaporated, diluted with water, and the product was extracted with EtOAc. The extract was washed with brine, evaporated, and dried. To a solution of the reaction mixture in 3 mL of DMF was added sodium azide (10 mg, 0.16 mmol). The reaction mixture was stirred at 100 °C for 12 h, and then concentrated. The residue was dissolved in EtOAc and the organic phase was evaporated and dried. To a solution of residue in MeOH/CH₂Cl₂ (3/1, 3 mL) was added 10% Pd/C (50 wt %). The reaction mixture was hydrogenated at rt for 12 h. The catalyst was removed by filtration through a pad of Celite, which was rinsed with MeOH/CH₂Cl₂ (3/1). The residue was washed with EtOAc/hexane (1/1), evaporated, and dried, affording RB-033 as a white solid; ¹H NMR (400 MHz, CD₃OD) δ 0.89 (t, J = 6.9 Hz, 3H), 1.27–1.32 (m, 18H), 1.58–1.62 (m, 2H), 2.00–2.10 (m, 2H), 2.29 (d, J = 13.2, 2H), 2.58–2.62 (m, 2H), 3.05–3.09 (m, 2H), 3.15 (d, J = 13.6 Hz, 2H), 3.28–3.33 (m, 2H), 3.46–3.54 (m, 1H), 3.71 (d, J = 10.9 Hz, 2H), 7.17 (d, J = 7.9 Hz, 2H), 7.22 (d, J = 7.9 Hz, 2H); ¹³C NMR (100 MHz, CD₃OD) δ 14.5, 23.8, 29.9, 30.3, 30.5, 30.6, 30.7, 30.8, 30.9, 31.3, 32.8, 33.1, 36.5, 36.9, 129.9, 130.1, 133.1, 143.1; ESI-HRMS (M + H)⁺ m/z calcd for C₂₅H₄₅N₂ 373.3583, found 373.3576.
**Preparation of 4-Fluoro-1-(4-octylphenethyl)piperidine (RB-034)**

To a solution of **RB-005** (12 mg, 0.040 mmol) in CH$_2$Cl$_2$ (3 mL) at 0 °C was added diethylaminosulfur trifluoride (DAST, 15 µL, 0.12 mmol). After being stirred at rt for 5 h, the reaction mixture was diluted with water, and the product was extracted with EtOAc. The extract was washed with brine, evaporated, and dried. Purification by silica gel chromatography, eluting with CH$_2$Cl$_2$/MeOH (10:1), gave 11 mg (90%) of **RB-034** as a colorless oil; $^1$H NMR (400 MHz, CDCl$_3$) δ 0.87 (t, $J = 6.8$ Hz, 3H), 1.25–1.30 (m, 10H), 1.54–1.62 (m, 2H), 1.90–2.00 (m, 4H), 2.48–2.63 (m, 6H), 2.76–2.80 (m, 2H), 4.61–4.66 (m, 0.5H), 4.74–4.78 (m, 0.5H), 7.10 (s, 4H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 14.1, 22.7, 29.3, 29.4, 29.5, 29.7, 33.2, 35.6, 49.4, 60.5, 128.5, 137.2, 140.8; ESI-HRMS (M + H)$^+$ m/z calcd for C$_{21}$H$_{35}$FN 320.2754, found 320.2751.

**Preparation of 1-(4-Octylphenethyl)piperidin-4-one (RB-035)**

To a solution of **RB-005** (25 mg, 0.080 mmol) in CH$_2$Cl$_2$ (3 mL) at 0 °C was added pyridinium chlorochromate (PCC, 25 mg, 0.12 mmol). After being stirred at rt for 4 h, the reaction mixture was diluted with water, and the product was extracted with EtOAc. The extract was washed with brine, evaporated, and dried. Purification by silica gel chromatography, eluting with CH$_2$Cl$_2$/MeOH (3:1), gave 17 mg (70%) of **RB-035** as a colorless oil; $^1$H NMR (400 MHz, CDCl$_3$) δ 0.87 (t, $J = 6.6$ Hz, 3H), 1.22–1.30 (m, 10H), 1.58–1.60 (m, 2H), 2.47–2.52 (m, 4H), 2.57 (t, $J = 7.7$ Hz, 2H), 2.72–2.74 (m, 2H),
Preparation of 4-Methoxy-1-(4-octylphenethyl)piperidine (RB-036)

To a solution of 4-octylphenethyl methanesulfonate (17 mg, 50 µmol) in MeCN (3 mL) was added at rt. After the suspension was stirred for 10 min, 4-methoxypiperidine (19 mg, 0.16 mmol) was added. The reaction mixture was stirred at 50 °C for 12 h. The solvent was evaporated and the residue was purified by silica gel chromatography, eluting with CH₂Cl₂/MeOH (5:1), to give 14 mg (79%) of RB-036 as a yellow liquid; ¹H NMR (400 MHz, CDCl₃) δ 0.87 (t, J = 6.5 Hz, 3H), 1.23–1.31 (m, 10H), 1.56–1.61 (m, 2H), 1.67–1.72 (m, 2H), 1.95–2.00 (m, 2H), 2.30–2.37 (m, 2H), 2.56 (t, J = 7.7 Hz, 2H), 2.62–2.64 (m, 2H), 2.79–2.85 (m, 4H), 3.25–3.30 (m, 1H), 3.34 (s, 3H), 7.10 (s, 4H); ¹³C NMR (100 MHz, CDCl₃) δ 14.1, 22.7, 29.3, 29.4, 29.5, 29.7, 31.6, 31.9, 33.7, 35.6, 36.0, 41.2, 53.1, 59.4, 128.6, 137.0, 141.0, 178.0; ESI-HRMS (M + H)⁺ m/z calcd for C₂₁H₃₄NO 316.2640, found 316.2635.

Preparation of (S)-1-(4-Octylphenethyl)pyrrolidin-3-ol (RB-037)

To a solution of 4-octylphenethyl methanesulfonate (20 mg, 0.064 mmol) in MeCN (4 mL), K₂CO₃ (44 mg, 0.32 mmol) was added at rt. After the suspension was stirred for 10 min, (S)-pyrrolidine-3-ol hydrochloride (79 mg, 0.64 mmol) was added. The reaction mixture was stirred at 50 °C for 12 h. The reaction mixture was diluted with
water, and the product was extracted with EtOAc. The extract was washed with brine, dried, and evaporated. Purification by silica gel chromatography, eluting with CH$_2$Cl$_2$/MeOH (3:1), gave 17 mg (86%) of **RB-037** as a yellow liquid; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 0.88 (t, $J = 6.8$ Hz, 3H), 1.22–1.32 (m, 10H), 1.58 (quin, $J = 7.3$ Hz, 2H), 1.85 (quin, $J = 6.7$ Hz, 1H), 2.19–2.28 (m, 1H), 2.46–2.54 (m, 2H), 2.56 (t, $J = 7.8$ Hz, 2H), 2.68 (dd, $J = 5.1$, 10.4 Hz, 1H), 2.78–2.88 (m, 4H), 2.91 (d, $J = 10.4$ Hz, 1H), 3.07–3.13 (m, 1H), 3.30–3.35 (m, 1H), 4.47–4.50 (m, 1H), 7.12 (s, 4H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 14.1, 22.6, 29.3, 29.4, 29.5, 31.5, 31.9, 33.4, 34.2, 35.6, 52.9, 57.9, 62.7, 70.4, 128.5, 128.7, 135.2, 141.5; ESI-HRMS (M + H)$^+$ m/z calcd for C$_{20}$H$_{34}$NO 304.2640, found 304.2637.

Preparation of (R)-1-(4-Octylphenethyl)pyrrolidin-3-ol (RB-038)

![RB-038](image)

Compound **RB-038** was prepared from 4-octylphenethyl methanesulfonate according to a coupling procedure similar to that described for **RB-037**, using (R)-pyrrolidine; yield = 72%; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 0.88 (t, $J = 6.7$ Hz, 3H), 1.26–1.29 (m, 10H), 1.56–1.59 (m, 2H), 1.94–2.01 (m, 1H), 2.22–2.31 (m, 1H), 2.56 (t, $J = 7.9$ Hz, 2H), 2.74–2.76 (m, 1H), 2.91–2.99 (m, 4H), 3.14–3.24 (m, 2H), 3.30–3.35 (m, 1H), 4.47–4.50 (m, 1H), 7.12 (s, 4H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 14.1, 22.6, 29.3, 29.4, 29.5, 31.5, 31.9, 33.4, 34.2, 35.6, 52.9, 57.9, 62.7, 70.4, 128.5, 128.7, 135.2, 141.5; ESI-HRMS (M + H)$^+$ m/z calcd for C$_{20}$H$_{34}$NO 304.2640, found 304.2637.

Preparation of (R)-(1-(4-Methylphenethyl)pyrrolidin-2-yl)methanol (RB-039)

![RB-039](image)

Compound **RB-039** was prepared from 2-(4-methylphenyl)ethanol according to a coupling procedure similar to that described for **RB-037**, using D-prolinol; yield = 62%;
\(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 1.84–1.91 (m, 1H), 1.97–1.21 (m, 1H), 2.03–2.13 (m, 2H), 2.31 (s, 3H), 2.83–2.89 (m, 1H), 2.96–3.08 (m, 2H), 3.13–3.21 (m, 1H), 3.34–3.41 (m, 1H), 3.49–3.56 (m, 1H), 3.72–3.77 (m, 1H), 3.85 (d, \(J = 2.31\) Hz, 2H), 2.83–2.89 (m, 1H), 2.96–3.08 (m, 2H), 3.13–3.21 (m, 1H), 3.34–3.41 (m, 1H), 3.49–3.56 (m, 1H), 3.72–3.77 (m, 1H), 3.85 (d, \(J = 5.3\) Hz, 2H), 7.12 (dd, \(J = 1.9, 8.4\) Hz, 4H); \(^13\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 21.0, 23.6, 26.5, 32.0, 39.4, 50.7, 55.0, 57.9, 61.1, 69.5, 128.6, 129.5, 133.7, 136.7; ESI-HRMS (M + H)\(^+\) m/z calc'd for C\(_{14}\)H\(_{22}\)NO

Preparation of (R)-(1-(4-Octylphenethyl)pyrrolidin-2-yl)methanol (RB-040)

Compound RB-040 was prepared from 4-octylphenethyl methanesulfonate according to a coupling procedure similar to that described for RB-037, using D-prolinol; yield = 59%; \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 0.88 (t, \(J = 6.8\) Hz, 3H), 1.22–1.31 (m, 10H), 1.54–1.62 (m, 2H), 1.84–2.05 (m, 4H), 2.56 (t, \(J = 7.8\) Hz, 2H), 2.64–2.70 (m, 1H), 2.85–3.00 (m, 3H), 3.08–3.13 (m, 1H), 3.32–3.36 (m, 1H), 3.57–3.62 (m, 1H), 3.67 (dd, \(J = 5.5, 13.7\) Hz, 1H), 3.78 (dd, \(J = 3.2, 12.2\) Hz, 1H), 7.11 (s, 4H); \(^13\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 14.1, 22.7, 23.8, 26.9, 29.3, 29.5, 29.7, 31.0, 31.5, 31.9, 35.6, 54.5, 56.9, 61.4, 66.3, 128.5, 128.7, 135.5, 141.5; ESI-HRMS (M + H)\(^+\) m/z calc'd for C\(_{21}\)H\(_{36}\)NO 318.2797, found 318.2792.

Preparation of (S)-(1-(4-Octylphenethyl)pyrrolidin-2-yl)methanol (RB-041)

Compound RB-041 was prepared from 4-octylphenethyl methanesulfonate according to a coupling procedure similar to that described for RB-037, using L-prolinol; yield = 54%; \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 0.88 (t, \(J = 6.7\) Hz, 3H), 1.22–1.31 (m, 10H), 1.58 (quin, \(J = 7.3\) Hz, 2H), 1.80–1.96 (m, 4H), 2.52–2.55 (m, 1H), 2.56 (t, \(J = 7.8\) Hz,
Preparation of (R)-(1-(4-Dodecylphenethyl)pyrrolidin-2-yl)methanol (RB-042)

Compound RB-042 was prepared from 2-(4-dodecylphenyl)ethanol according to a coupling procedure similar to that described for RB-037, using D-prolinol; yield = 62%;

\[
\text{\textsuperscript{1}H NMR (400 MHz, CDCl}_3) \delta 0.87 (t, J = 6.6 Hz, 3H), 1.22–1.31 (m, 18H), 1.56–1.60 (m, 2H), 2.00–2.16 (m, 4H), 2.56 (t, J = 7.7 Hz, 2H), 2.82–2.88 (m, 1H), 3.03–3.09 (m, 2H), 3.22–3.30 (m, 1H), 3.35–3.39 (m, 1H), 3.46–3.53 (m, 1H), 3.75–3.80 (m, 1H), 3.86 (dd, J = 6.5, 12.7 Hz, 1H), 3.95 (dd, J = 2.1, 12.0 Hz, 1H), 7.12 (s, 4H); \text{\textsuperscript{13}C NMR (100 MHz, CDCl}_3) \delta 14.1, 22.7, 24.0, 26.5, 29.3, 29.4, 29.5, 29.6, 29.7, 31.5, 31.8, 31.9, 35.5, 54.7, 58.0, 60.9, 70.2, 128.5, 128.9, 133.6, 142.0; ESI-HRMS (M + H)}^+ m/z calcd for C\textsubscript{25}H\textsubscript{44}NO 374.3423, found 374.3418.
\]

Preparation of (S)-(1-(4-Dodecylphenethyl)pyrrolidin-2-yl)methanol (RB-043)

Compound RB-043 was prepared from 2-(4-dodecylphenyl)ethanol according to a coupling procedure similar to that described for RB-037, using L-prolinol; yield = 55%;

\[
\text{\textsuperscript{1}H NMR (400 MHz, CDCl}_3) \delta 0.88 (t, J = 6.6 Hz, 3H), 1.23–1.30 (m, 18H), 1.56–1.62 (m, 2H), 1.93–2.11 (m, 4H), 2.56 (t, J = 7.8 Hz, 2H), 2.98–3.06 (m, 2H), 3.14–3.22 (m, 1H), 3.28–3.32 (m, 1H), 3.41–3.49 (m, 1H), 3.65–3.68 (m, 1H), 3.71–3.73 (m, 1H), 3.79–
Preparation of Compounds RB-044 – RB-050

These products were prepared from 4-iodobenzoic acid in three steps. First, 4-(oct-1-ynyl)benzoic acid was prepared by a Sonogashira reaction. To a deaerated solution of 4-iodobenzoic acid (500 mg, 2.02 mmol), bis(triphenylphosphine)palladium dichloride (116 mg, 0.10 mmol), and copper(I) iodide (19 mg, 0.10 mmol) in anhydrous triethylamine (15 mL) was added 1-octyne (0.89 mL, 6.05 mmol) at rt. The reaction mixture was heated at 60 °C for 12 h. After saturated aqueous ammonium chloride solution was added, the product was extracted with EtOAc. The combined solution was washed with water, brine, and dried. Purification by silica gel chromatography, eluting with hexane/EtOAc (3:1), gave 395 mg (85%) of the alkyne product as a yellow liquid; $^1$H NMR (400 MHz, CDCl$_3$) δ 0.90 (t, $J$ = 6.7 Hz, 3H), 1.31–1.35 (m, 4H), 1.43–1.50 (m, 2H), 1.58–1.66 (m, 2H), 2.43 (t, $J$ = 7.1 Hz, 2H), 7.47 (d, $J$ = 8.4 Hz, 2H), 8.02 (d, $J$ = 8.4 Hz, 2H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 13.8, 19.3, 22.3, 28.3, 31.1, 79.8, 94.4, 127.6, 129.6, 129.7, 131.3, 171.8; negative-ion ESI-HRMS (M - H)$^-$ m/z calcd for C$_{15}$H$_{17}$O$_2^-$ 229.1234, found 229.1237. Then, catalytic hydrogenation afforded 4-octylbenzoic acid as a yellow solid without purification; $^1$H NMR (400 MHz, CDCl$_3$) δ 0.88 (t, $J$ = 6.8 Hz, 3H), 1.22–1.32 (m, 10H), 1.61–1.64 (m, 2H), 2.64 (t, $J$ = 7.7 Hz, 2H), 7.25 (d, $J$ = 8.1 Hz, 2H), 8.02 (d, $J$ = 8.2 Hz, 2H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 14.1, 22.7, 29.3, 29.4, 31.1, 31.9, 36.1, 126.9, 128.6, 130.3, 149.6, 172.6; negative-ion ESI-HRMS (M - H)$^-$ m/z calcd for C$_{15}$H$_{21}$O$_2^-$ 233.1547, found 233.1548.
Preparation of (R)-(4-Dodecylphenyl)-(2-(hydroxymethyl)pyrrolidin-1-yl)methanone (RB-044)

To a solution of 4-(n-dodecyl)benzoic acid (10 mg, 0.034 mmol) in CH$_2$Cl$_2$ (3 mL), thionyl chloride (0.25 mL, 0.34 mmol) was added at rt. The reaction mixture was heated at reflux for 12 h. The reaction mixture was diluted with water, and the product was extracted with EtOAc. The extract was washed with brine, dried, and evaporated. To a solution of residue in MeCN (3 mL) was added K$_2$CO$_3$ (24 mg, 0.17 mmol) at rt. After the suspension was stirred for 10 min, D-prolinol (10 mg, 0.10 mmol) was added. The reaction mixture was stirred at 50 °C for 12 h. The reaction mixture was diluted with water, and the product was extracted with EtOAc. The extract was washed with brine, dried, and evaporated. Purification by silica gel chromatography, eluting with CH$_2$Cl$_2$/MeOH (10:1), gave 9 mg (72%) of **RB-044** as a yellow liquid; $^1$H NMR (400 MHz, CDCl$_3$) δ 0.88 (t, $J$ = 6.6 Hz, 3H), 1.23–1.34 (m, 18H), 1.59–1.64 (m, 4H), 1.70–1.77 (m, 1H), 1.84–1.89 (m, 1H), 2.14–2.21 (m, 1H), 2.62 (t, $J$ = 7.6 Hz, 2H), 3.46–3.59 (m, 2H), 3.71–3.82 (m, 2H), 4.39–4.44 (m, 1H), 7.20 (d, $J$ = 7.9 Hz, 2H), 7.43 (d, $J$ = 7.9 Hz, 2H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 14.1, 22.7, 25.1, 28.6, 29.2, 29.3, 29.4, 29.5, 29.6, 29.7, 31.3, 31.9, 35.8, 51.3, 61.6, 67.6, 127.2, 128.3, 133.8, 145.5, 172.5; ESI-HRMS (M + H)$^+$ m/z calcd for C$_{24}$H$_{40}$NO$_2$ 374.3059, found 374.3055.

Preparation of (S)-(4-Dodecylphenyl)(2-(hydroxymethyl)pyrrolidin-1-yl)methanone (RB-045)
Compound **RB-045** was prepared from 4-((n-dodecyl)benzoic acid according to a coupling procedure similar to that described for **RB-044**, using L-prolinol instead of D-prolinol; yield = 65%; $^1$H NMR (400 MHz, CDCl$_3$) δ 0.88 (t, $J = 6.7$ Hz, 3H), 1.23–1.30 (m, 18H), 1.58–1.64 (m, 4H), 1.70–1.77 (m, 1H), 1.84–1.89 (m, 1H), 2.14–2.21 (m, 1H), 2.62 (t, $J = 7.7$ Hz, 2H), 3.47–3.59 (m, 2H), 3.71–3.82 (m, 2H), 4.38–4.44 (m, 1H), 7.20 (d, $J = 8.0$ Hz, 2H), 7.43 (d, $J = 7.9$ Hz, 2H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 14.1, 22.7, 25.1, 28.6, 29.2, 29.4, 29.5, 29.6, 29.7, 31.2, 31.9, 35.8, 51.3, 61.6, 67.5, 127.2, 128.3, 133.8, 145.5, 172.5; ESI-HRMS (M + H)$^+$ m/z calcd for C$_{24}$H$_{40}$NO$_2$ 374.3059, found 374.3058.

**Preparation of (4-Hydroxypiperidin-1-yl)(4-octylphenyl)methanone (RB-046)**

![Structure RB-046]

Compound **RB-046** was prepared from 4-((n-dodecyl)benzoic acid according to a coupling procedure similar to that described for **RB-044**, using 4-hydroxypiperidine; yield = 70%; $^1$H NMR (400 MHz, CDCl$_3$) δ 0.88 (t, $J = 6.7$ Hz, 3H), 1.26–1.30 (m, 10H), 1.51–1.62 (m, 4H), 1.80–1.97 (m, 2H), 2.63 (t, $J = 7.7$ Hz, 2H), 3.21–3.36 (m, 2H), 3.67–3.76 (m, 1H), 3.93–4.00 (m, 1H), 4.18–4.26 (m, 1H), 7.19 (d, $J = 8.2$ Hz, 2H), 7.31 (d, $J = 8.2$ Hz, 2H), $^{13}$C NMR (100 MHz, CDCl$_3$) δ 14.1, 22.7, 29.2, 29.3, 29.4, 31.3, 35.8, 67.4, 126.9, 128.5, 130.2, 133.2, 144.8, 170.7; ESI-HRMS (M + H)$^+$ m/z calcd for C$_{29}$H$_{32}$NO$_2$ 318.2428, found 318.2432.

**Preparation of (4-Dodecylphenyl)(4-hydroxypiperidin-1-yl)methanone (RB-047)**

![Structure RB-047]
Compound **RB-047** was prepared from 4-(*n*-dodecyl)benzoic acid according to a coupling procedure similar to that described for **RB-044**, using 4-hydroxypiperidine; yield = 81%; $^1$H NMR (400 MHz, CDCl$_3$) δ 0.88 (t, $J = 6.7$ Hz, 3H), 1.22–1.37 (m, 18H), 1.58–1.63 (m, 4H), 1.84–1.95 (m, 2H), 2.61 (t, $J = 7.7$ Hz, 2H), 3.21–3.34 (m, 2H), 3.67–3.75 (m, 1H), 3.95 (sep, $J = 3.9$ Hz, 1H), 4.18–4.23 (m, 1H), 7.19 (d, $J = 7.9$ Hz, 2H), 7.30 (d, $J = 8.0$ Hz, 2H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 14.2, 22.7, 29.3, 29.4, 29.5, 29.6, 29.7, 31.3, 31.9, 35.8, 67.2, 126.9, 128.5, 133.1, 144.9, 170.8; ESI-HRMS (M + H)$^+$ m/z calcd for C$_{24}$H$_{40}$NO$_2$ 374.3059, found 374.3055.

**Preparation of 4-Octyl-N-(pyridin-4-ylmethyl)benzamide (RB-048)**

![Structure of RB-048]

Compound **RB-048** was prepared from 4-(*n*-dodecyl)benzoic acid according to a coupling procedure similar to that described for **RB-044**, using 4-(aminomethyl)pyridine; yield = 69%; $^1$H NMR (400 MHz, CDCl$_3$) δ 0.88 (t, $J = 6.8$ Hz, 3H), 1.07–1.30 (m, 10H), 1.59–1.63 (m, 2H), 2.65 (t, $J = 7.7$ Hz, 2H), 4.63 (d, $J = 6.0$ Hz, 2H), 6.97 (t, $J = 5.6$ Hz, NH), 7.24 (d, $J = 8.0$ Hz, 4H), 7.74 (d, $J = 8.2$ Hz, 2H), 8.51–8.56 (m, 2H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 14.1, 21.1, 22.7, 29.2, 29.4, 29.7, 31.2, 31.8, 35.8, 42.7, 60.4, 127.1, 128.5, 128.7, 131.2, 147.4, 147.8, 149.9, 167.7, 171.2; ESI-HRMS (M + H)$^+$ m/z calcd for C$_{21}$H$_{29}$N$_2$O 325.2274, found 325.2277.

**Preparation of N-(4-Hydroxyphenyl)-4-octylbenzamide (RB-049)**

![Structure of RB-049]
Compound RB-049 was prepared from 4-(n-dodecyl)benzoic acid according to a coupling procedure similar to that described for RB-044, using 4-aminophenol; yield = 55%; \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 0.88 (t, \(J = 6.8\) Hz, 3H), 1.11–1.29 (m, 10H), 1.54–1.60 (m, 2H), 2.67 (t, \(J = 7.1\) Hz, 2H), 6.83 (d, \(J = 7.8\) Hz, 2H), 7.28 (d, \(J = 7.8\) Hz, 2H), 7.43 (d, \(J = 6.8\) Hz, 2H), 7.80 (d, \(J = 6.8\) Hz, 2H); \(^1\)^3^C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 14.3, 22.9, 27.6, 29.6, 29.7, 30.0, 30.4, 31.6, 32.2, 36.2, 123.2, 127.5, 129.0, 132.5, 147.6, 167.8; ESI-HRMS (M + H\(^+\)) \textit{m/z} calcd for C\(_{21}\)H\(_{28}\)NO\(_2\) 326.2115, found 326.2118.

**Preparation of N-(4-(2-Hydroxyethyl)phenyl)-4-octylbenzamide (RB-050)**

![Chemical Structure of RB-050](image)

Compound RB-050 was prepared from 4-(n-dodecyl)benzoic acid according to a coupling procedure similar to that described for RB-044, using 2-(4-aminophenyl)ethanol; yield = 73%; \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 0.88 (t, \(J = 6.8\) Hz, 3H), 1.24–1.32 (m, 10H), 1.51–1.60 (m, 2H), 2.67 (t, \(J = 7.7\) Hz, 2H), 2.87 (t, \(J = 6.5\) Hz, 2H), 3.86 (t, \(J = 6.5\) Hz, 2H), 7.23 (d, \(J = 8.4\) Hz, 2H), 7.29 (d, \(J = 8.1\) Hz, 2H), 7.58 (d, \(J = 8.4\) Hz, 2H), 7.76 (br s, NH), 7.78 (d, \(J = 8.1\) Hz, 2H); \(^1\)^3^C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 14.1, 22.7, 27.3, 29.2, 29.4, 29.7, 31.2, 31.9, 38.6, 63.7, 120.5, 127.0, 128.9, 129.7, 132.3, 134.6, 136.5, 147.4, 165.7; ESI-HRMS (M + Na\(^+\)) \textit{m/z} calcd for C\(_{23}\)H\(_{31}\)NO\(_2\)Na 376.2247, found 376.2251.

**Preparation of RB-051 and RB-052**

These compounds were prepared from 1-bromo-4-iodobenzene. First, 1-bromo-4-(oct-1-ynyl)benzene was prepared from 1-bromo-4-iodobenzene by a Sonogashira reaction; yield = 70%; \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 0.90 (t, \(J = 6.5\) Hz, 3H), 1.29–1.34 (m, 4H), 1.40–1.47 (m, 2H), 1.59 (quin, \(J = 7.3\) Hz, 2H), 2.37 (t, \(J = 7.1\) Hz, 2H), 7.24 (dt, \(J = 8.3, 2.0\) Hz, 2H), 7.39 (dt, \(J = 8.6, 2.0\) Hz, 2H); \(^1\)^3^C NMR (100 MHz, CDCl\(_3\)) \(\delta\)
Preparation of 4-(4-Octylphenethyl)pyridine (RB-051)

To a deaerated solution of 1-bromo-4-(oct-1-ynyl)benzene (100 mg, 0.38 mmol), bis(triphenylphosphine)palladium dichloride (22 mg, 0.010 mmol), and copper(I) iodide (4 mg, 10 µmol) in anhydrous triethylamine (8 mL) was added 3-ethynylpyridine (78 mg, 0.75 mmol) at rt. The reaction mixture was heated at 80 °C for 3 d. After saturated ammonium chloride solution was added, the product was extracted with EtOAc. The combined solution was washed with water, brine, and dried. The catalyst was removed by filtration through a pad of Celite, which was rinsed with hexanes/EtOAc (3:1). 3-((4-Oct-1-ynyl)phenyl)ethynyl)pyridine (53 mg, 0.18 mmol) was dissolved in EtOAc (8 mL), and 10% Pd/C (53 mg, 100 wt %) was added. The reaction mixture was hydrogenated at rt for 12 h. The catalyst was removed by filtration through a pad of Celite, which was rinsed with EtOAc. Flash column chromatography with hexanes/EtOAc (1:1) as eluent gave RB-051 (48 mg, 88%) as a yellow oil; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 0.88 (t, $J$ = 6.8 Hz, 3H), 1.27–1.34 (m, 10H), 1.59 (quin, $J$ = 7.3 Hz, 2H), 2.56 (t, $J$ = 7.8 Hz, 2H), 2.86–2.92 (m, 4H), 7.05 (d, $J$ = 8.1 Hz, 2H), 7.09 (d, $J$ = 8.1 Hz, 2H), 7.17 (dd, $J$ = 4.8, 7.7 Hz, 1H), 7.43 (dt, $J$ = 7.8, 1.8 Hz, 1H), 8.42-8.44 (m, 2H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 14.2, 22.7, 29.3, 29.4, 29.5, 31.6, 31.9, 35.0, 35.6, 37.1, 123.2, 128.3, 128.5, 135.9, 137.0, 140.8, 147.5, 150.0; ESI-HRMS (M + H)$^+$ m/z calcd for C$_{21}$H$_{30}$N 296.2378, found 296.2374.

14.1, 19.5, 22.6, 28.8, 31.4, 79.6, 91.8, 121.5, 123.1, 131.4, 133.0; ESI-HRMS (M)$^+$ m/z calcd for C$_{14}$H$_{17}$Br 264.0514, found 264.0508.
Preparation of 1-Methyl-4-(4-octylphenethyl)pyridinium Iodide (RB-052)

![Chemical Structure](image)

To a solution of **RB-051** (32 mg, 0.11 mmol) in MeCN (5 mL) was added K$_2$CO$_3$ (75 mg, 0.54 mmol). After the suspension was stirred at rt for 10 min, MeI (30 µL, 0.54 mmol) was added. The reaction mixture was stirred overnight at rt. The reaction mixture was diluted with water, and the product was extracted with EtOAc. The extract was washed with brine, dried, and evaporated. The residue was washed with hexane to give 38 mg (80%) of **RB-052** as a yellow solid; $^1$H NMR (400 MHz, CDCl$_3$) δ 0.88 (t, $J$ = 6.9 Hz, 3H), 1.26–1.30 (m, 10H), 1.56 (quin, $J$ = 7.3 Hz, 2H), 2.54 (t, $J$ = 7.8 Hz, 2H), 3.02 (t, $J$ = 7.6 Hz, 2H), 3.17 (t, $J$ = 7.6 Hz, 2H), 4.60 (s, 3H), 7.07 (dd, $J$ = 6.9 Hz, 1H), 8.06 (d, $J$ = 8.0 Hz, 1H), 9.12 (d, $J$ = 6.5 Hz, 2H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 14.1, 22.7, 29.3, 29.4, 29.5, 31.6, 31.9, 34.4, 35.5, 35.7, 49.2, 127.6, 128.6, 128.7, 136.1, 141.4, 142.8, 143.0, 145.1, 145.2; ESI-HRMS (M)$^+$ m/z calcd for C$_{22}$H$_{32}$N$^+$ 310.2535, found 310.2533.

Preparation of 4-(4-Methylpiperidin-1-yl)-1-(4-octylbenzyl)pyridinium bromide (RB-053)

![Chemical Structure](image)

First, 1-(bromomethyl)-4-(oct-1-ynyl)benzene was prepared from 4-iodobenzyl bromide and 1-octyne by a Sonogashira reaction; yield = 69%; $^1$H NMR (400 MHz, CDCl$_3$) δ 0.87 (t, $J$ = 6.9 Hz, 3H), 1.27–1.30 (m, 8H), 2.38 (t, $J$ = 6.8 Hz, 2H), 4.44 (s, 2H), 7.30 (d, $J$ = 8.0 Hz, 2H), 7.33 (d, $J$ = 8.0 Hz, 2H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ
14.1, 19.3, 22.4, 28.4, 28.7, 31.2, 33.3, 80.0, 91.2, 124.3, 128.9, 131.8, 136.9; ESI-HRMS (M + H)\(^+\) \textit{m/z} calcd for C\(_{15}\)H\(_{20}\)Br 279.0748, found 279.0730. Then, this compound was subjected to N-alkylation with 4-(4-methylpiperidin-1-yl)pyridine. The latter compound was synthesized from 4-chloropyridine hydrochloride (0.20 g, 1.3 mmol) in dry acetonitrile (4 mL). \(N,N\)-Diisopropylethylamine (DIPEA, 0.7 mL, 4.0 mmol) was added, followed by 4-methylpiperidine (0.16 mL, 1.3 mmol). The reaction mixture was subjected to microwave irradiation at 160 °C for 1 h. After the reaction mixture was cooled rt, EtOAc was added, and the solution was washed with water and brine, dried (Na\(_2\)SO\(_4\)), and concentrated in vacuo. Ether (3 mL) was added to the resulting crude oil, and the inorganic precipitate was removed by filtration. Evaporation of the solvents afforded 4-(4-methylpiperidin-1-yl)pyridine. To a solution of 1-(bromomethyl)-4-(oct-1-ynyl)benzene (100 mg, 0.35 mmol) in 5 mL of 2-butanone was added 4-(4-methylpiperidin-1-yl)pyridine (124 mg, 0.71 mmol) in a sealed tube. The reaction mixture was stirred at 100 °C for 3 d and concentrated. The residue was washed with EtOAc to give 125 mg (78%) of 4-(4-methylpiperidin-1-yl)-1-(4-(oct-1-ynyl)benzyl)pyridinium bromide (\textbf{RB-053}) as a slightly yellow solid; \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 0.86 (t, \(J = 7.0\) Hz, 3H), 0.98 (d, \(J = 6.4\) Hz, 3H), 1.16–1.33 (m, 10H), 1.55–1.60 (m, 4H), 1.72–1.78 (m, 1H), 1.84 (d, \(J = 13.8\) Hz, 2H), 2.58 (t, \(J = 7.6\) Hz, 2H), 3.12 (td, \(J = 12.5, 2.4\) Hz, 2H), 4.08 (d, \(J = 13.5\) Hz, 2H), 5.47 (s, 2H), 7.02 (d, \(J = 7.6\) Hz, 2H), 7.18 (d, \(J = 8.0\) Hz, 2H), 7.43 (d, \(J = 8.0\) Hz, 2H), 8.46 (d, \(J = 7.6\) Hz, 2H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 14.2, 19.4, 21.3, 22.6, 28.6, 29.7, 30.5, 31.3, 33.5, 47.4, 60.3, 79.8, 92.2, 108.4, 125.3, 128.9, 132.4, 133.1, 143.0, 155.2; ESI-HRMS (M + H)\(^+\) \textit{m/z} calcd for C\(_{26}\)H\(_{36}\)N\(_2\)\(^+\) 376.2873, found 376.2871. Catalytic hydrogenation provided 4-(4-methylpiperidin-1-yl)-1-(4-octylbenzyl)pyridinium bromide (\textbf{RB-053}) in 87% yield; \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 0.86 (t, \(J = 7.0\) Hz, 3H), 0.98 (d, \(J = 6.4\) Hz, 3H), 1.16–1.33 (m, 10H), 1.55–1.60 (m, 4H), 1.72–1.78 (m, 1H), 1.84 (d, \(J = 13.8\) Hz, 2H), 2.58 (d, \(J = 7.6\) Hz, 2H), 3.13 (td, \(J = 12.5, 2.4\) Hz, 2H), 4.08 (d, \(J = 13.5\) Hz, 2H), 5.47 (s, 2H), 7.02 (d, \(J = 7.6\) Hz, 2H), 7.18 (d, \(J = 8.0\) Hz, 2H), 7.34 (d, \(J = 8.0\) Hz, 2H), 8.46 (d, \(J = 7.5\) Hz, 2H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 14.1, 21.3, 22.7, 29.2, 29.3, 29.4, 29.7, 30.3, 30.5, 31.4, 31.9, 33.5, 35.7, 47.5, 60.8, 108.5, 128.8, 129.5, 130.9, 142.8, 144.5, 155.2; ESI-HRMS (M)\(^+\) \textit{m/z} calcd for C\(_{26}\)H\(_{39}\)N\(_2\)+ 379.3113, found 379.3108.
Preparation of 3-(1-(4-Octylphenyl)-1H-1,2,3-triazol-4-yl)pyridine (RB-054)

![Chemical Structure](image)

This compound was prepared from 4-iodoaniline in three steps. First, 4-(oct-1-ynyl)aniline was prepared from 4-iodoaniline and 1-octyne; yield = 62%; \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 0.90 (t, \(J = 7.0\) Hz, 3H), 1.26–1.35 (m, 4H), 1.40–1.47 (m, 2H), 1.58 (quin, \(J = 7.4\) Hz, 2H), 2.37 (t, \(J = 7.1\) Hz, 2H), 6.57 (d, \(J = 8.6\) Hz, 2H), 7.19 (d, \(J = 8.6\) Hz, 2H); \(^13\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 14.1, 19.5, 22.6, 28.6, 29.0, 31.4, 80.7, 87.9, 113.7, 114.8, 132.7, 145.9; ESI-HRMS (M + H)\(^+\) m/z calcd for C\(_{14}\)H\(_{20}\)N 202.1596, found 202.1589. The aryl amine was converted to the corresponding aryl azide by the reaction of 4-(oct-1-ynyl)aniline (157 mg, 0.78 mmol) in 2 mL of 10% aqueous HCl with NaNO\(_2\) (65 mg, 0.94 mmol) in 1 mL of water at 0 °C. After the solution was stirred for 30 min, NaN\(_3\) (61 mg, 0.94 mmol) in 1 mL of water was added at 0 °C, with stirring for another hour. The reaction mixture was warmed to 25 °C, diluted with EtOAc, washed with water and brine, dried (Na\(_2\)SO\(_4\)), and concentrated in vacuo, affording the aryl azide. Then, a Cu(I)-catalyzed azide-alkyne 1,3-dipolar (click) reaction was carried out. Without purification, the aryl azide (43 mg, 0.19 mmol) and 3-ethynlypyridine (39 mg, 0.38 mmol) were dissolved in t-BuOH/H\(_2\)O (3 mL, 1:1), and CuSO\(_4\) (30 mg, 0.19 mmol) and sodium ascobate (37 mg, 0.19 mmol) were added at rt. The reaction mixture was stirred for 2 days and then was diluted with EtOAc and washed with brine. The aqueous layer was extracted with EtOAc. The combined organic layers were dried (MgSO\(_4\)) and concentrated in vacuo. Purification by silica gel chromatography, eluting with hexanes/EtOAc (1:1), gave 50 mg (67%, 2 steps) of 3-(1-(4-(oct-1-ynyl)phenyl-1H-1,2,3-triazol-4-yl)pyridine as a white solid; \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 0.92 (t, \(J = 6.9\) Hz, 3H), 1.29–1.38 (m, 4H), 1.47 (quin, \(J = 7.3\) Hz, 2H), 1.63 (quin, \(J = 7.3\) Hz, 2H), 2.44 (t, \(J = 7.1\) Hz, 2H), 7.41 (dd, \(J = 4.8, 7.9\) Hz, 1H), 7.57 (d, \(J = 8.6\) Hz, 2H), 7.74 (d, \(J = 8.6\) Hz, 2H), 8.27–8.30 (m, 2H), 8.61–8.63 (m, 1H), 9.08 (s, 1H); \(^13\)C NMR (100 MHz,
CDCl$_3$ δ 14.1, 19.5, 22.6, 28.5, 28.6, 31.4, 79.3, 93.0, 117.8, 120.2, 123.1, 123.9, 125.2, 126.4, 133.0, 133.3, 135.6, 145.4, 147.1, 149.6, 153.2, ESI-HRMS (M + H)$^+$ m/z calcld for C$_{21}$H$_{23}$N$_4$ 331.1923, found 331.1919. Catalytic hydrogenation afforded 3-(1-(4-octylphenyl)-1H-1,2,3-triazol-4-yl)pyridine (RB-054) in 82% yield; $^1$H NMR (400 MHz, CDCl$_3$) δ 0.89 (t, $J$ = 6.8 Hz, 3H), 1.25–1.35 (m, 10H), 1.62–1.68 (m, 6H), 2.69 (t, $J$ = 7.7 Hz, 2H), 7.36 (d, $J$ = 8.3 Hz, 2H), 7.42 (dd, $J$ = 4.8, 7.8 Hz, 1H), 7.69 (d, $J$ = 8.3 Hz, 2H), 8.25 (s, 1H), 8.30 (d, $J$ = 7.9 Hz, 1H), 8.61–8.63 (m, 1H), 9.08 (s, 1H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 14.1, 22.7, 29.2, 29.3, 29.4, 29.7, 31.4, 31.9, 35.5, 118.1, 120.6, 123.9, 126.6, 129.8, 133.2, 134.7, 144.4, 145.2, 147.1, 149.4; ESI-HRMS (M + H)$^+$ m/z calcld for C$_{21}$H$_{27}$N$_4$ 335.2236, found 335.2232.

**Preparation of 4-(4-Octyl-1H-1,2,3-triazol-1-yl)phenol (RB-055)**

![RB-055](Image)

4-(4-Octyl-1H-1,2,3-triazol-1-yl)phenol (RB-055) was prepared from 4-aminophenol according to a procedure similar to that described in the above click reaction; yield = 70%; $^1$H NMR (400 MHz, CDCl$_3$) δ 0.86 (t, $J$ = 6.7 Hz, 3H), 1.23–1.38 (m, 10H), 1.72 (t, $J$ = 8.6 Hz, 2H), 2.79 (t, $J$ = 8.5 Hz, 2H), 7.11 (d, $J$ = 7.6 Hz, 2H), 7.54 (d, $J$ = 7.6 Hz, 2H), 7.68 (s, 1H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 14.1, 22.6, 25.5, 29.2, 29.3, 29.4, 31.8, 116.7, 119.6, 122.3, 129.7, 148.8, 157.7; ESI-HRMS (M + H)$^+$ m/z calcld for C$_{16}$H$_{24}$N$_3$O 274.1914, found 274.1918.

**Preparation of 2-(4-(4-Octyl-1H-1,2,3-triazol-1-yl)phenyl)ethanol (RB-056)**

![RB-056](Image)
2-(4-(4-Octyl-1H,1,2,3-triazol-1-yl)phenyl)ethanol (RB-056) was prepared by a click reaction as follows. To a solution of 4-(azidophenyl)-2-ethanol (200 mg, 1.23 mmol) and 1-decyne (508 mg, 3.68 mmol) in tert-BuOH/H₂O (6 mL, 1:1) were added CuSO₄ (196 mg, 1.23 mmol) and sodium ascorbate (243 mg, 1.23 mmol). The reaction mixture was stirred at rt for 12 h and then was diluted with EtOAc and washed with brine. The aqueous layer was extracted with EtOAc. The combined organic layers were dried (MgSO₄) and concentrated in vacuo. Purification by silica gel chromatography, eluting with CH₂Cl₂/MeOH (10:1), gave 296 mg (80%) of RB-056 as a white solid; ¹H NMR (400 MHz, CDCl₃) δ 0.88 (t, J = 6.9 Hz, 3H), 1.27–1.42 (m, 10H), 1.71 (quin, J = 7.5 Hz, 2H), 2.76 (t, J = 7.7 Hz, 2H), 2.92 (t, J = 6.6 Hz, 2H), 3.89 (t, J = 6.6 Hz, 2H), 7.35 (d, J = 8.5 Hz, 2H), 7.61 (d, J = 8.5 Hz, 2H), 7.70 (s, 1H); ¹³C NMR (100 MHz, CDCl₃) δ 14.1, 22.6, 25.6, 29.1, 29.2, 29.3, 29.4, 31.8, 38.7, 63.1, 118.9, 120.4, 130.2, 135.6, 139.7, 149.1; ESI-HRMS (M + H)⁺ m/z calcd for C₁₈H₂₈N₃O 302.2227, found 302.2230.

Preparation of RB-057 via Triazole Intermediates.

4-(4-Butyl-1H,1,2,3-triazol-1-yl)phenethyl Methanesulfonate

To a solution of 4-(azidophenyl)-2-phenethyl alcohol (200 mg, 1.23 mmol) and 1-hexyne (0.42 mL, 3.68 mmol) in tert-BuOH/H₂O (6 mL, 1:1) were added CuSO₄ (196 mg, 1.23 mmol) and sodium ascorbate (243 mg, 1.23 mmol). The reaction mixture was stirred at rt for 12 h and then was diluted with EtOAc and washed with brine. The aqueous layer was extracted with EtOAc. The combined organic layers were dried (MgSO₄) and concentrated in vacuo, affording the triazole without purification, as a yellow liquid. To a solution of triazole (1.23 mmol) and triethylamine (0.86 mL, 6.15 mmol) in CH₂Cl₂ (10 mL) at 0 °C was added methanesulfonyl chloride (0.29 mL, 3.69 mmol). After being stirred at rt for 5 h, the reaction mixture was evaporated, diluted with water, and the product was extracted with EtOAc. The extract was washed with brine,
dried, and evaporated. Purification by silica gel chromatography, eluting with hexanes/EtOAc (1:2), gave 253 mg (66%, 2 steps) of 4-(4-butyl-1H-1,2,3-triazol-1-yl)phenethyl methanesulfonate as a white solid; \( ^1H \) NMR (400 MHz, CDCl\(_3\)) \( \delta \) 0.96 (t, \( J = 7.3 \) Hz, 3H), 1.43 (sex, \( J = 7.5 \) Hz, 2H), 1.72 (quin, \( J = 7.6 \) Hz, 2H), 2.80 (t, \( J = 7.7 \) Hz, 2H), 2.92 (s, 3H), 3.12 (t, \( J = 6.7 \) Hz, 2H), 4.45 (t, \( J = 6.7 \) Hz, 2H), 7.38 (d, \( J = 8.5 \) Hz, 2H), 7.69 (d, \( J = 8.5 \) Hz, 2H), 7.73 (s, 1H); \( ^{13}C \) NMR (100 MHz, CDCl\(_3\)) \( \delta \) 13.9, 22.3, 25.3, 31.5, 31.6, 35.1, 37.4, 69.7, 118.8, 120.6, 130.3, 136.3, 136.9, 149.2; ESI-HRMS (M + H)\(^+\) m/z calcd for C\(_{15}\)H\(_{22}\)N\(_3\)O\(_3\)S 324.1382, found 324.1382.

**Preparation of 4-(4-Pentyl-1H-1,2,3-triazol-1-yl)phenethyl Methanesulfonate**

This compound was prepared according to a coupling procedure similar to that described for 4-(4-butyl-1H-1,2,3-triazol-1-yl)phenethyl methanesulfonate, using 1-heptyne; yield = 58% (2 steps); \( ^1H \) NMR (400 MHz, CDCl\(_3\)) \( \delta \) 0.91 (t, \( J = 7.1 \) Hz, 3H), 1.36–1.40 (m, 4H), 1.74 (quin, \( J = 7.5 \) Hz, 2H), 2.79 (t, \( J = 7.7 \) Hz, 2H), 2.93 (s, 3H), 3.12 (t, \( J = 6.7 \) Hz, 2H), 4.46 (t, \( J = 6.7 \) Hz, 2H), 7.38 (d, \( J = 8.5 \) Hz, 2H), 7.69 (d, \( J = 8.5 \) Hz, 2H), 7.74 (s, 1H); \( ^{13}C \) NMR (100 MHz, CDCl\(_3\)) \( \delta \) 14.0, 22.4, 25.6, 29.1, 31.4, 31.6, 35.1, 37.4, 60.4, 69.7, 118.8, 120.6, 130.3, 136.2, 136.9, 149.3; ESI-HRMS (M + H)\(^+\) m/z calcd for C\(_{16}\)H\(_{24}\)N\(_3\)O\(_3\)S 338.1538, found 338.1536.

**Preparation of 1-(4-(4-Butyl-1H-1,2,3-triazol-1-yl)phenethyl)piperidine (RB-057)**

![RB-057](image)

To a solution of 4-(4-pentyl-1H-1,2,3-triazol-1-yl)phenethyl methanesulfonate (50 mg, 0.15 mmol) in 3 mL of acetonitrile was added piperidine (150 \( \mu \)L, 1.54 mmol). The reaction mixture was stirred at 50 °C for 12 h and concentrated. Purification by silica gel chromatography, eluting with CH\(_2\)Cl\(_2\)/MeOH (5:1), gave 11 mg (75%) of RB-057 as a slightly yellow waxy solid; \( ^1H \) NMR (400 MHz, CDCl\(_3\)) \( \delta \) 0.96 (t, \( J = 7.4 \) Hz, 3H), 1.42
(sex, $J = 7.4$ Hz, 2H), 1.51–1.55 (m, 2H), 1.72 (quin, $J = 7.6$ Hz, 2H), 1.80 (quin, $J = 5.4$ Hz, 4H), 2.74–2.83 (m, 8H), 3.04–3.08 (m, 2H), 7.36 (d, $J = 8.4$ Hz, 2H), 7.63 (d, $J = 8.4$ Hz, 2H), 7.71 (s, 1H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 13.9, 22.3, 23.5, 24.7, 25.3, 29.7, 31.5, 31.9, 54.1, 60.7, 118.8, 120.5, 129.9, 135.6, 140.6, 149.1; ESI-HRMS (M + H)$^+$ m/z calcd for C$_{19}$H$_{29}$N$_4$ 313.2392, found 313.2385.

**Preparation of 1-(4-(4-Pentyl-1H-1,2,3-triazol-1-yl)phenethyl)piperidine (RB-058)**

![Image of RB-058]

Compound RB-058 was prepared from 27 according to a coupling procedure similar to that described for RB-057; yield = 81%; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 0.91 (t, $J = 6.9$ Hz, 3H), 1.36–1.43 (m, 4H), 1.47–1.52 (m, 2H), 1.67–1.77 (m, 6H), 2.53–2.61 (m, 4H), 2.65–2.69 (m, 2H), 2.78 (d, $J = 7.7$ Hz, 2H), 2.92–2.96 (m, 2H), 7.34 (d, $J = 8.4$ Hz, 2H), 7.62 (d, $J = 8.4$ Hz, 2H), 7.69 (s, 1H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 14.0, 22.4, 24.0, 25.5, 25.7, 29.1, 31.5, 32.7, 54.4, 60.7, 118.8, 120.5, 129.9, 135.6, 140.6, 149.1; ESI-HRMS (M + H)$^+$ m/z calcd for C$_{20}$H$_{31}$N$_4$ 327.2549, found 327.2543.

**Preparation of 1-(4-(4-Octyl-1H-1,2,3-triazol-1-yl)phenethyl)piperidine (RB-059)**

![Image of RB-059]

To a solution of RB-056 (50 mg, 0.17 mmol) and triethylamine (116 µL, 0.83 mmol) in CH$_2$Cl$_2$ (5 mL) at 0 °C was added methanesulfonyl chloride (39 µL, 0.51 mmol). After being stirred at rt for 5 h, the reaction mixture was evaporated, diluted with
water, and the product was extracted with EtOAc. The extract was washed with brine, dried, and evaporated to afford the mesylate as a yellow liquid. To a solution of 65 mg (0.17 mmol) of the mesylate (without purification) in 3 mL of acetonitrile was added piperidine (168 µL, 1.70 mmol). The reaction mixture was stirred at 50 °C for 12 h and concentrated. Purification by silica gel chromatography, eluting with CH₂Cl₂/MeOH (5:1), gave 11 mg (66%) of RB-059 as a slightly yellow waxy solid; ¹H NMR (400 MHz, CDCl₃) δ 0.88 (t, J = 6.8 Hz, 3H), 1.25–1.39 (m, 10H), 1.58–1.62 (m, 2H), 1.72 (quin, J = 7.3 Hz, 2H), 1.89 (quin, J = 5.1 Hz, 4H), 2.78 (t, J = 7.7 Hz, 2H), 2.96–3.04 (m, 6H), 3.14–3.18 (m, 2H), 7.40 (d, J = 8.1 Hz, 2H), 7.64 (d, J = 8.1 Hz, 2H), 7.67 (s, 1H); ¹³C NMR (100 MHz, CDCl₃) δ 14.0, 22.6, 22.8, 23.9, 25.6, 29.1, 29.2, 29.3, 30.9, 31.8, 53.8, 59.1, 118.9, 120.6, 130.0, 135.8, 138.5, 149.1, 173.3; ESI-HRMS (M + H)⁺ m/z calcd for C₂₃H₃₇N₄ 369.3013, found 369.3015; (M + H - N₂)⁺ m/z calcd for C₂₃H₃₅N₂ 341.2951, found 341.2954.

Preparation of 1-(4-(4-Butyl-1H-1,2,3-triazol-1-yl)phenethyl)-1-methylpiperidinium Methanesulfonate (RB-060)

To a solution of 4-(4-butyl-1H-1,2,3-triazol-1-yl)phenethyl methanesulfonate (15 mg, 46 µmol) in 3 mL of acetonitrile was added 1-methylpiperidine (23 mg, 0.23 mmol). The reaction mixture was stirred at 50 °C for 12 h and concentrated. The residue was washed with hexane to give 16 mg (82%) of RB-060 as a yellow oil; ¹H NMR (400 MHz, CDCl₃) δ 0.95 (t, J = 7.3 Hz, 3H), 1.41 (sex, J = 7.5 Hz, 2H), 1.66–1.74 (m, 4H), 1.80–1.87 (m, 4H), 2.77 (s, 3H), 2.74–2.84 (m, 2H), 3.13–3.17 (m, 2H), 3.57 (t, J = 5.5 Hz, 4H), 3.71–3.76 (m, 2H), 7.54 (d, J = 8.5 Hz, 2H), 7.62 (d, J = 8.5 Hz, 2H), 7.76 (s, 1H); ¹³C NMR (100 MHz, CDCl₃) δ 13.9, 20.2, 20.8, 21.5, 22.3, 22.8, 25.3, 27.8, 31.5, 39.6,
44.2, 55.4, 61.0, 119.1, 120.6, 120.7, 136.1, 136.2, 149.2; ESI-HRMS (M)^+ m/z calcd for C_{20}H_{31}N_{4}^+ 327.2549, found 327.2546.

**Preparation of 1-Methyl-1-(4-(4-octyl-1H-1,2,3-triazol-1-yl)phenethyl)piperidinium Methanesulfonate (RB-061)**

![RB-061](image)

Compound **RB-061** was prepared according to a coupling procedure similar to that described for **RB-060**; yield = 77%; ^1^H NMR (400 MHz, CDCl_3) δ 0.90 (t, J = 7.2 Hz, 3H), 1.34–1.39 (m, 4H), 1.68–1.75 (m, 4H), 1.81–1.86 (m, 4H), 2.76 (s, 3H), 2.74–2.83 (m, 2H), 3.13–3.18 (m, 2H), 3.28 (s, 3H), 3.59 (t, J = 5.5 Hz, 4H), 3.75–3.79 (m, 2H), 7.55 (d, J = 8.5 Hz, 2H), 7.64 (d, J = 8.5 Hz, 2H), 7.76 (s, 1H); ^13^C NMR (100 MHz, CDCl_3) δ 14.0, 20.2, 20.8, 21.5, 22.4, 22.8, 25.6, 27.9, 29.1, 31.5, 39.7, 44.2, 55.3, 61.0, 119.0, 120.6, 120.8, 130.3, 130.8, 136.0, 136.2, 149.3; ESI-HRMS (M)^+ m/z calcd for C_{21}H_{33}N_{4}^+ 341.2705, found 341.2704.

**Preparation of 1-Methyl-1-(4-(4-octyl-1H-1,2,3-triazol-1-yl)phenethyl)piperidinium methanesulfonate (RB-062)**

![RB-062](image)

Compound **RB-062** was prepared according to a coupling procedure similar to that described for **RB-060**; yield = 71%; ^1^H NMR (400 MHz, CDCl_3) δ 0.87 (t, J = 7.1 Hz, 3H), 1.27–1.49 (m, 10H), 1.71–1.78 (m, 4H), 1.85–1.89 (m, 4H), 2.76 (s, 3H), 2.80–2.90 (m, 2H), 3.14–3.18 (m, 2H), 3.35 (s, 3H), 3.48 (t, J = 5.5 Hz, 4H), 3.67–3.85 (m, 2H), 7.60 (d, J = 8.5 Hz, 2H), 7.68 (d, J = 8.5 Hz, 2H), 7.83 (s, 1H); ^13^C NMR (100 MHz,
CDCl$_3$ δ 14.2, 20.2, 20.5, 20.9, 21.4, 22.7, 23.0, 25.7, 27.9, 28.9, 29.1, 29.3, 29.4, 29.5, 31.9, 39.6, 44.0, 55.0, 61.1, 119.1, 120.4, 120.8, 130.2, 130.8, 136.1, 136.3, 149.3; ESI-HRMS (M)$^+$ m/z calcd for C$_{24}$H$_{39}$N$_4$ + 383.3169, found 383.3174.

**Preparation of 1-(4-(4-Pentyl-1H-1,2,3-triazol-1-yl)phenethyl)piperidin-4-ol (RB-063)**

![Diagram of RB-063]

Compound RB-063 was prepared according to a coupling procedure similar to that described for RB-057, using 4-hydroxypiperidine; yield = 65%; $^1$H NMR (400 MHz, CDCl$_3$) δ 0.96 (t, J = 7.4 Hz, 3H), 1.42 (sex, J = 7.4 Hz, 2H), 1.60–1.75 (m, 4H), 1.93–1.97 (m, 2H), 2.26 (t, J = 9.4 Hz, 2H), 2.60–2.65 (m, 2H), 2.79 (t, J = 7.7 Hz, 2H), 2.85–2.89 (m, 4H), 3.75 (sep, J = 4.4 Hz, 1H), 7.33 (d, J = 8.4 Hz, 2H), 7.62 (d, J = 8.4 Hz, 2H), 7.69 (s, 1H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 13.9, 22.3, 25.3, 29.7, 31.5, 33.3, 34.4, 51.1, 60.0, 67.6, 118.8, 120.4, 129.9, 135.5, 141.0, 149.1; ESI-HRMS (M + H)$^+$ m/z calcd for C$_{19}$H$_{29}$N$_4$O 329.2341, found 329.2336.

**Preparation of 1-(4-(4-Pentyl-1H-1,2,3-triazol-1-yl)phenethyl)piperidin-4-ol (RB-064)**

![Diagram of RB-064]

Compound RB-064 was prepared according to a coupling procedure similar to that described for RB-057, using 4-hydroxypiperidine; yield = 70%; $^1$H NMR (400 MHz, CDCl$_3$) δ 0.90 (t, J = 7.1 Hz, 3H), 1.34–1.42 (m, 4H), 1.61–1.77 (m, 4H), 1.93–1.97 (m, 2H), 2.26 (t, J = 9.5 Hz, 2H), 2.61–2.65 (m, 2H), 2.78 (t, J = 7.7 Hz, 2H), 2.85–2.89 (m, 4H), 3.75 (sep, J = 4.2 Hz, 1H), 7.33 (d, J = 8.4 Hz, 2H), 7.62 (d, J = 8.4 Hz, 2H), 7.69
(s, 1H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 14.0, 22.4, 25.6, 29.1, 31.4, 33.3, 34.4, 51.0, 60.0, 67.6, 118.8, 120.4, 129.9, 135.5, 141.0, 149.1; ESI-HRMS (M + H)$^+$ m/z calcd for C$_{20}$H$_{31}$N$_4$O 343.2498, found 343.2495.

**Preparation of 1-(4-(4-Octyl-1H-1,2,3-triazol-1-yl)phenethyl)piperidin-4-amine (RB-065).**

Compound RB-065 was prepared according to a coupling procedure similar to that described for RB-057, using 4-hydroxypiperidine; yield = 63%; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 0.87 (t, $J$ = 6.7 Hz, 3H), 1.27–1.39 (m, 12H), 1.71 (quin, $J$ = 7.5 Hz, 2H), 1.77–1.84 (m, 2H), 2.77 (t, $J$ = 7.7 Hz, 4H), 2.91–2.95 (m, 2H), 3.05–3.07 (m, 2H), 3.09–3.14 (m, 2H), 3.89–3.93 (m, 1H), 7.37 (d, $J$ = 8.4 Hz, 2H), 7.63 (d, $J$ = 8.4 Hz, 2H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 14.1, 22.6, 25.6, 29.1, 29.2, 29.3, 29.4, 31.7, 50.0, 58.9, 118.9, 120.6, 130.0, 135.8, 138.9, 149.2; ESI-HRMS (M + H)$^+$ m/z calcd for C$_{23}$H$_{37}$N$_4$O 385.2962, found 385.2965.
Preparation of (S,E)-3-azido-3-(hydroxymethyl)-5-(4-octylphenyl)pent-1-enylphosphonic acid and (S,E)-3-azido-3-(fluoromethyl)-5-(4-octylphenyl)pent-1-enylphosphonic acid

Scheme 5. Preparation of (S,E)-3-azido-3-(hydroxymethyl)-5-(4-octylphenyl)pent-1-enylphosphonic acid and (S,E)-3-azido-3-(fluoromethyl)-5-(4-octylphenyl)pent-1-enylphosphonic acid

Data for (S)-2-(4-octylphenethyl)oxirane-2-carbaldehyde: $^1$H NMR (500 MHz, CDCl$_3$) δ 0.83-0.92 (m, 3H), 1.22-1.36 (m, 10H), 1.56-1.64 (m, 2H), 2.01-2.08 (m, 1H), 2.20-2.28 (m, 1H), 2.58 (t, $J$ = 7.8 Hz, 2H), 2.72 (t, $J$ = 8.2 Hz, 2H), 2.99 (d, $J$ = 4.6 Hz, 1H), 3.04 (d, $J$ = 4.6 Hz, 1H), 7.10-7.13 (m, 4H), 8.89 (s, 1H); $^{13}$C NMR (125 MHz, CDCl$_3$) δ 14.1, 22.6, 29.2, 29.3, 29.5, 29.9, 30.2, 31.5, 31.9, 35.5, 49.8, 60.9, 128.1, 128.5, 138.0, 140.8, 198.8; ESI-HRMS (M + Na)$^+$ m/z calcd for C$_{19}$H$_{28}$NaO$_2$ + 311.1982, found 311.1986.

(E)-Dimethyl 2-[(R)-2-(4-octylphenethyl)oxiran-2-yl]vinylphosphonate. To a mixture of NaH (57-63% oil dispersion, 240 mg, 6.00 mmol) and 50 mL of THF was added a solution of 1.41 g (6.08 mmol) of CH$_2$[(P(O)(OMe)$_2$)] in 10 mL of THF at 0 °C. After the
mixture had been stirred for 30 min, a solution of (S)-2-(4-octylphenethyl)oxirane-2-carbaldehyde (585 mg, 2.03 mmol) in 10 mL of THF was added. The reaction mixture was stirred for 1 h at 0 °C, quenched with saturated aqueous NH$_4$Cl solution, and extracted with EtOAc. The organic layer was dried (MgSO$_4$) and concentrated under reduced pressure. The residue was purified by chromatography (elution with EtOAc) to give 750 mg (94%) of the desired dimethyl phosphonate product. $^1$H NMR (400 MHz, CDCl$_3$) δ 0.83-0.92 (m, 3H), 1.20-1.38 (m, 10H), 1.53-1.64 (m, 2H), 1.90-2.01 (m, 1H), 2.08-2.18 (m, 1H), 2.53-2.59 (m, 2H), 2.62-2.77 (m, 3H), 2.87 (t, $J$ = 5.4 Hz, 1H), 3.72 (d, $J$ = 5.5 Hz, 3H), 3.74 (d, $J$ = 5.5 Hz, 3H), 5.95 (dd, $J$ = 17.2, 19.4 Hz, 1H), 6.83 (dd, $J$ = 17.2, 22.2 Hz, 1H), 7.05-7.13 (m, 4H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 14.1, 22.6, 29.2, 29.3, 29.4, 30.6, 31.5, 31.8, 35.2, 35.5, 52.38 (d, $J$ = 5.4 Hz), 52.41 (d, $J$ = 5.4 Hz), 55.9, 58.2 (d, $J$ = 24.0 Hz), 116.5 (d, $J$ = 189.6 Hz), 128.0, 128.5, 137.9, 140.8, 151.6 (d, $J$ = 6.5 Hz); $^{31}$P NMR (162 MHz, CDCl$_3$) δ 20.6; ESI-HRMS (M + H)$^+$ m/z calcld for C$_{22}$H$_{36}$O$_4$P$^+$ 395.2346, found 395.2346.

$(S,E)$-Dimethyl 3-azido-3-(hydroxymethyl)-5-(4-octylphenyl)pent-1-enylphosphonate. Ti(O-i-Pr)$_4$ (2.4 mL, 8.02 mmol) and TMSN$_3$ (2.2 mL, 16.6 mmol) were added to anhydrous toluene (50 mL), and the mixture was heated at reflux (85 °C) under N$_2$ for at least 5 h. A solution of the epoxide (670 mg, 1.70 mmol) in anhydrous toluene (10 mL) was added to the above solution in one portion. The mixture was stirred for 15 min at 85 °C and was then cooled to rt. The solvent was removed under reduced pressure. Et$_2$O (20 mL) was added, followed by 10% HCl (40 mL). The solution was stirred at rt until two clear phases appeared. The aqueous phase was extracted with Et$_2$O. The organic phase was dried over MgSO$_4$, filtered, and concentrated under reduced pressure. Purification by flash chromatography (elution with EtOAc) afforded the target 3-azido-3-hydroxymethyl dimethyl phosphonate ester (500 mg, 68%) as a yellow oil. $^1$H NMR (500 MHz, CDCl$_3$) δ 0.83-0.92 (m, 3H), 1.20-1.36 (m, 10H), 1.54-1.62 (m, 2H), 1.90-1.99 (m, 1H), 2.03-2.11 (m, 1H), 2.51-2.67 (m, 4H), 3.01 (s, 1H, OH), 3.70-3.80 (m, 8H), 6.03 (dd, $J$ = 17.1, 19.3 Hz, 1H), 6.72 (dd, $J$ = 17.2, 22.7 Hz, 1H), 7.06-7.12 (m, 4H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 14.1, 22.6, 29.2, 29.3, 29.46, 29.50, 31.6, 31.9, 35.5,
(S,E)-3-Azido-3-(hydroxymethyl)-5-(4-octylphenyl)pent-1-enylphosphonic acid. To a solution of the dimethyl phosphonate ester (10 mg, 0.023 mmol) in 2 mL of dry CH₂Cl₂ at rt was added 30 mL (0.23 mmol) of TMSBr. After the reaction mixture had been stirred for 6 h, the solvent was removed, and the residue was dried and dissolved in 2 mL of 95% MeOH with stirring for 1 h. Removal of the solvent afforded 9 mg (100%) of the product. ¹H NMR (500 MHz, CDCl₃) δ 0.83-0.92 (m, 3H), 1.20-1.36 (m, 10H), 1.52-1.63 (m, 2H), 1.85-1.94 (m, 1H), 1.97-2.07 (m, 1H), 2.52-2.68 (m, 4H), 3.67-3.74 (m, 2H), 6.09-6.22 (m, 1H), 6.45-6.59 (m, 1H), 7.07-7.13 (m, 4H); ¹³C NMR (125 MHz, CDCl₃) δ 13.7, 22.4, 28.98, 29.04, 29.1, 29.2, 29.4, 31.3, 31.6, 35.2, 36.0, 68.4 (d, J = 18.4 Hz), 127.8, 128.4, 138.1, 140.5, 146.7; ³¹P NMR (202 MHz, CDCl₃) δ 15.5; ESI-HRMS (M + H)⁺ m/z calcd for C₂₂H₃₆N₃O₄P⁺ 438.2516, found 438.2519.

(S,E)-Dimethyl 3-Azido-3-(fluoromethyl)-5-(4-octylphenyl)pent-1-enylphosphonate. To a solution of alcohol (S,E)-dimethyl 3-azido-3-(hydroxymethyl)-5-(4-octylphenyl)pent-1-enylphosphonate (75 mg, 0.171 mmol) in anhydrous CH₂Cl₂ (1.0 mL) was added diethylaminosulfur trifluoride (DAST) (68 mL, 0.515 mmol) at -78 °C. The reaction mixture was stored at -78 °C overnight, and then was stirred at rt for 3 h. The mixture was poured into aqueous saturated NaHCO₃ solution, the aqueous phase was extracted with CH₂Cl₂, and the combined CH₂Cl₂ layers were dried (Na₂SO₄), filtered, and concentrated under reduced pressure. Purification by flash chromatography (elution with PhMe/EtOAc, 1:1 to 100% EtOAc) afforded 23 (49 mg, 65%), along with 10 mg (13%) of recovered alcohol. ¹H NMR (500 MHz, CDCl₃) δ 0.83-0.92 (m, 3H), 1.20-1.36 (m, 10H), 1.53-1.63 (m, 2H), 1.88-2.02 (m, 1H), 2.12-2.22 (m, 1H), 2.51-2.60 (m, 3H), 2.64-2.72 (m, 1H), 3.34-3.53 (m, 2H), 3.77 (d, J = 11.1 Hz, 6H), 6.12 (dd, J = 17.1, 18.8, 1H), 6.73 (ddd, J = 17.1, 21.4, 22.8 Hz, 1H), 7.03-7.13 (m, 4H); ¹³C NMR (100 MHz,
**ASSAYS**

**Cell Culture.** HEK 293 cells stably over-expressing GFP-SK1 (30-fold increase in SK1 activity versus vector-transfected cells) were cultured in DMEM supplemented with 10% European fetal calf serum, 100 U/mL penicillin, 100 μg/mL streptomycin, 1% nonessential amino acids, and 0.8% geneticin at 37 °C in 5% CO₂.

**Sphingosine Kinase Activity Assays.** In order to measure SK2 activity, sphingosine (Sph) was complexed with fatty acid free bovine serum albumin (final concentration, 0.2 mg/mL) in buffer A containing 20 mM Tris (pH 7.4), 1 mM EDTA, 1 mM Na₃VO₄, 40 mM β-glycerophosphate, 1 mM NaF, 0.007% (v/v) β-mercaptoethanol, 20% (v/v) glycerol, 10 μg/mL aprotinin, 10 μg/mL soybean trypsin inhibitor, 1 mM PMSF, 0.5 mM...

CDCl₃ δ 14.1, 22.6, 28.6, 28.7, 29.2, 29.3, 29.4, 31.5, 31.9, 35.5, 37.4 (d, J = 21.9 Hz), 52.5 (t, J = 5.5 Hz), 56.5 (d, J = 23.9 Hz), 97.5 (dd, J = 19.6, 184.9 Hz), 118.0 (dd, J = 9.6, 187.8 Hz), 128.0, 128.6, 137.5, 141.0, 149.5 (dd, J = 5.8, 20.4 Hz); 3¹P NMR (162 MHz, CDCl₃) δ 19.6; ¹⁹F NMR (376 MHz, CDCl₃) δ −164.5; ESI-HRMS (M + H)⁺ m/z calcd for C₂₂H₃₆FN₃O₃P⁺ 440.2473, found 440.2472.

(S,E)-3-Azido-3-(fluoromethyl)-5-(4-octylphenyl)pent-1-enylphosphonic acid. To a solution of (S,E)-dimethyl 3-azido-3-(fluoromethyl)-5-(4-octylphenyl)pent-1-enylphosphonate (10 mg, 0.023 mmol) in 2 mL of dry CH₂Cl₂ at rt was added 30 μL (0.23 mmol) of TMSBr. After the reaction mixture had been stirred for 6 h, the solvent was removed, and the residue was dried and dissolved in 2 mL of 95% MeOH with stirring for 1 h. Removal of the solvent afforded 10 mg (100%) of the product as a white solid. ¹H NMR (400 MHz, CDCl₃/CD₃OD/CDCl₂ 80:20:1) δ 0.83-0.92 (m, 3H), 1.20-1.36 (m, 10H), 1.52-1.63 (m, 2H), 1.87-2.05 (m, 1H), 2.10-2.24 (m, 1H), 2.50-2.69 (m, 4H), 3.40-3.50 (m, 2H), 6.16-6.28 (m, 1H), 6.49-6.68 (m, 1H), 7.04-7.14 (m, 4H); ¹³C NMR (125 MHz, CDCl₃/CD₃OD/CDCl₂ 80:20:1) δ 13.7, 22.3, 28.3, 29.0, 29.2, 31.3, 31.6, 35.2, 37.1, 37.3, 56.3 (d, J = 24.4 Hz), 97.2 (d, J = 184.2 Hz), 127.8, 128.3, 137.7, 140.6, 145.2; ³¹P NMR (162 MHz, CDCl₃/CD₃OD/CDCl₂ 80:20:1) δ 15.2; ESI-HRMS (M + H)⁺ m/z calcd for C₂₀H₂₅FNO₃P⁺ 386.2255, found 386.2256.
4-deoxypyridoxine, and 400 mM KCl. SK2 assays were performed using 37 ng of purified SK2 and incubating the assay for 30 min at 30 °C in the presence of 10 µM Sph, 250 µM [γ-32P]ATP in 10 mM MgCl₂, and varying concentrations of the inhibitors dissolved in DMSO or control (5% v/v DMSO). To measure SK1 activity, Sph was solubilized in Triton X-100 (final concentration, 0.063% w/v) and combined with buffer A without KCl. 30 µg of recombinant SK1 was incubated for 30 min at 30 °C, in the presence of 3 µM Sph, 250 µM [γ-32P]ATP in 10 mM MgCl₂ with or without inhibitor dissolved in DMSO or control (5% v/v DMSO). Both assay reactions were terminated by the addition of 500 µL of 1-butanol. After 1 mL of 2 M KCl was added, with mixing, two phases were formed. The lower (aqueous) phase, which contains unreacted [γ-32P]ATP, was removed and discarded. The organic phase containing [32P]-S1P was extracted by washing twice with 2 M KCl (1 mL each time) before quantification by Cerenkov counting. To evaluate the test compounds as putative substrates of SK1 and SK2, the assay was conducted in the presence of 50 µM of the test compound (but in the absence of Sph) and radioactivity in the 1-butanol phase was quantified.
WE CLAIM:

1.) A compound having formula I:

\[ \text{(I)} \]

wherein

- \( R^1 \) is a hydrogen, lower alkyl, or lower alkoxy;
- \( R^2 \) and \( R^3 \) are independently hydrogen, \( C_{1-10} \) alkyl, or \(-C(X)NHAr\);

wherein

- \( X \) is oxygen or sulfur;
- \( Ar \) is aryl or heteroaryl group;
- \( R^4 \) is a hydrogen, or hydroxyl;
- \( R^5 \) is a \( C_{mH_{2m+1}} \) straight-chain or branched alkyl, \( C_{2-20} \)-alkenyl, \( C_{2-20} \)-alkynyl, or \( C_{1-20} \)-alkoxy;

\( m \) is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20;
wherein bond A is a single bond or a double bond;
with the proviso that when \( R^2 \) and \( R^3 \) are hydrogen, \( R^1 \) is not methyl; and
with the proviso that when \( A \) is a double bond, \( R^1 \) is not hydroxymethyl.

2.) The compound according to claim 1, wherein the heteroaryl group is mono- or poly-halophenyl.

3.) The compound according to claim 1, selected from the group consisting of:

\[ \text{, and } \]

\[ \text{; } \]
4.) The compound according to claim 1, selected from the group consisting of:

wherein X is sulfur or oxygen.
5.) A compound having formula II:

(II)

\[
\begin{array}{c}
\text{X} \\
\text{Y} \\
\text{R}^1 \\
\text{n} \\
\text{W} \\
\text{Z} \\
\text{OH} \\
\text{N}_3 \\
\text{P} \\
\text{O} \\
\text{OH} \\
\text{O} \\
\text{N}_3 \\
\text{Z}
\end{array}
\]

wherein

5. \( R^1 \) is C₃-C₁₂ alkyl, aryl, cycloalkyl, heterocyclyl, or heteroaryl, having a single cyclic ring or multiple condensed rings, quaternary ammonium group, or

\[
R^2 \text{ is straight-chain or branched alkyl } C_mH_{2m+1}, \ C_2-C_20-\text{alkenyl, } C_2-C_20-\text{alkynyl, } C_1-C_20-\text{alkoxy, or } C_2-C_20-\text{alkyl- substituted heterocycle;}
\]

\[
m \text{ is } 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, \text{ or } 20;
\]

\[
n \text{ is } 0, 1, 2, 3, 4, \text{ or } 5;
\]

15. \( W \) is –CH₂ or oxygen; and
X and Y are independently hydrogen, C₁-C₄-alkyl, or X and Y taken together are oxygen or sulfur.

6.) The compound according to claim 5, wherein R¹ is selected from the group consisting of:
wherein

n is 0, 1, 2, 3, 4, or 5; and
R is C₃-C₇-alkyl.

7.) The compound according to claim 5, wherein the heterocycle is selected from the group consisting of triazole, oxadiazole, oxazole, and thiazole.

8.) The compound according to claim 5, selected from the group consisting of:
wherein

10  R is hydrogen or hydroxyl; m is 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15; and

n is 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.
9.) A compound according to claim 5, wherein said compound comprises:

\[
R \text{ is a } C_mH_{2m+1} \text{ straight-chain or branched alkyl, } C_2-C_{20}\text{-alkenyl, } C_2-C_{20}\text{-alkynyl, or } C_1-C_{20}\text{-alkoxy;} \]
\[
m \text{ is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20;} \]
\[
X \text{ is } –OH, F, Br, Cl, \text{ or } I; \]
\[
n \text{ is 0, 1, 2, 3, 4, 5, or 6; and} \]
\[
W \text{ is oxygen or carbon.} \]
10.) A compound having formula III:

(III)

wherein

R\textsuperscript{1} is a C\textsubscript{m}H\textsubscript{2m+1} straight-chain or branched alkyl, C\textsubscript{2}-C\textsubscript{20}-alkenyl, C\textsubscript{2}-C\textsubscript{20}-alkynyl, or C\textsubscript{1}-C\textsubscript{20}-alkoxy;

m is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20;

R\textsuperscript{2} is hydrogen, hydroxyl, or C\textsubscript{1}-C\textsubscript{20}-alkoxyl;

R\textsuperscript{3} is oxygen or sulfur;

R\textsuperscript{4} is aryl or heteroaryl; and

X and Y are independently NH or oxygen.

11.) The compound according to claim 10, wherein the heteroaryl group is mono- or poly-halophenyl.
12.) A compound according to claim 10, selected from the group consisting of:

```
     O
   / \  
  H   C_{14}H_{29} OH
    \   |
     \  |
     N-   
    /\   |
   NH   |
  /     |
\       |
```

13.) A method of selectively inhibiting SK1 in a cell, said method comprising administering the compound of claim 3 or claim 8 or claim 12 to said cell.

14.) A method of selectively inhibiting SK2 in a cell, said method comprising administering the compound of claim 4 to said cell.

15.) A method of selectively activating SK1 in a cell, said method comprising administering the compound of claim 9 to said cell.

16.) A method of inducing apoptosis in a cell, said method comprising administering the compound of claim 3, claim 8 or claim 12 to said cell.
17.) A method of selectively inhibiting SK1 in a cell, said method comprising administering the compound of formula IV:

\[
\text{(IV)}
\]

\[\text{OH} \quad \text{C}_{15}\text{H}_{31} \quad \text{NH}_2\], or cis-sphingosine to said cell.
**ABSTRACT**

Sphingosine 1-phosphate (S1P) is involved in hyper-proliferative diseases, such as cancer and vascular remodeling in pulmonary arterial hypertension. Inhibitors of sphingosine kinase 1 and 2 (SK1 and SK2), which catalyze the synthesis of S1P, may be useful anti-proliferative agents. We have synthesized a series of sphingosine-based inhibitors of SK1 and SK2. Also provided in this invention are compounds that activate SK1 which can be used in diseases such as fibrosis, where intracellular S1P is anti-fibrotic.
FIG. 2
FIG. 2 continued
FIG. 3
FIG. 3  
(CONTINUED)
**FIG. 8**

![Bar graph]  

**FIG. 9**

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